TOLERANCE OF VESICULAR-ARBUSCULAR (VA) MYCORRHIZAL FUNGI TO ALUMINUM AND ITS RELATION TO IMPROVEMENT OF NUTRITION AND NODULATION OF LEGUMES IN AN ACID MINERAL SOIL

By

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Studies were conducted to test the hypothesis that the generally observed difference in effectiveness of VA mycorrhizal fungi in acid mineral soils is related to Al tolerance, and to determine if Al-sensitive isolates can develop tolerance by acclimation.

Several VA mycorrhizal fungi were evaluated for Al tolerance based on spore germination and hyphal growth in soils with 12%, 37%, and 100% Al saturation. Isolates which varied in tolerance were further evaluated for effectiveness in enhancing nodulation, nutrition, and growth of legumes in 100% Al-saturated soil. Some sensitive isolates were acclimatized to Al by culturing them in soils with progressively increasing soil percent Al saturation, and their

germination and growth in 100% Al-saturated soil after acclimation were evaluated.

There were interspecific and intraspecific variations in Al tolerance of VA mycorrhizal fungi. *Glomus* species were sensitive except *Gl. manihot* LMNH980, an isolate indigenous to 100% Al-saturated soil. *Glomus mosseae* LMSS156, LMSS313, LMSS378 were extremely sensitive, while *Gl. etunicatum* LETC236, LETC329, LETC455, and *Gl. clarum* LCLR551 were moderately sensitive. *Scutellispora* and *Gigaspora* species had high tolerance. *Scutellispora heterogama* CHTG139, *S. calospora* CCLS348, and *S. pellucida* CPLC288 were moderately tolerant, while *Gigaspora gigantea* GGGT109, GGGT663, *Gi. margarita* GMRG185, and GMRG444 were highly tolerant.

Similar variations occurred in the ability of VA mycorrhizal fungi to colonize *Pueraria phaseoloides* and *Stylosanthes guianensis*, and in the level of root colonization produced. Mycorrhizal colonization improved N-P nutrition, nodulation, and growth of legumes including *Leucaena leucocephala* and *Centrosema pubescens*. *Glomus manihot* LMNH980 was the most effective isolate.

Spore germination and mycelial growth index of these fungi in 100% Al-saturated soil were often highly correlated with mycorrhizal colonization, N-P nutrition, nodulation, and growth of the legumes in an acid mineral soil.

A third of the Al-sensitive isolates were able to develop tolerance after acclimation, but spore germination and hyphal length were limited.

The results demonstrated that Al tolerance of VA mycorrhizal fungi affects the extent of host root colonization and, consequently, their effectiveness in improving N-P nutrition, nodulation, and growth of host legumes in acid soil. Moreover, the results indicated that development of Al tolerance by sensitive isolates is possible by acclimation.

CHAPTER 1
GENERAL INTRODUCTION

High-Aluminum Acid Mineral Soils

High-aluminum, acid, mineral soils are predominantly distributed in South America, Southeast Asia, Central Africa, and other tropical and sub-tropical areas of the world. Plant growth is greatly limited in these soils. The factors which may cause acid soil infertility are toxicities of H (Evans and Kamprath, 1970; Islam et al., 1980), Al (Sartain and Kamprath, 1975; De Carvalho et al., 1980; Farina et al., 1980; Friesen et al., 1980), and Mn (Abruna et al., 1975; Burwester et al.,1981), and deficiencies of Ca (Gonzales-Erico et al., 1979), Mg (Breland et al., 1965), Mo (Mortvedt, 1981), and P (Fox, 1978). These factors may singly or simultaneously affect plant growth depending on plant species or genotypes (Godo and Reisenauer, 1980), soil percent base saturation (Andrew and Norris, 1961), organic matter (Yuan, 1959; 1963), and hydroxy-Al (Kissel et al., 1971). Apparently, there is a considerable evidence that Al toxicity (Foy, 1974; McLean, 1976; Sanchez, 1976; Blue and Dantzman, 1977; Juo, 1977) and P deficiency (Olsen and Watanabe, 1957) are the most common and the most important growth-limiting factors in acid mineral soils. Normal crop development is frequently impossible in

these soils without P application. In some cases, P may even replace lime as an amendment by reducing Al toxicity through fixation of exchangeable Al (Coleman et al., 1960) and by slightly increasing the negative charge on variable charge surfaces (Parfitt, 1978). Although P fertilization may temporarily alleviate the deficiency, only a small proportion of the P supplied by fertilization is made available to plants. Applied phosphate is rapidly adsorbed and fixed on surfaces of variable charge, primarily Fe and Al oxides, which predominate in the clay fraction of acid mineral soils (Syers et al., 1971; Weaver et al., 1975; Juo and Fox, 1977). Phosphorus adsorption capacity of soils was found correlated with extractable Al (Williams et al., 1958; Lee and Bartlett, 1977). Fairly good correlations between P fixation and exchangeable Al have also been obtained (Syers et al., 1971; Udo and Uzu, 1972). It was reported that 1 meq of exchangeable Al per 100 g of soil may fix up to 102 ppm P upon hydrolysis (Coleman et al., 1960). Fixed phosphates are unavailable to plants (Fox and Searle, 1978). Thus, effective utilization of available forms of P, whether naturally occurring or supplied by fertilization, may significantly improve the P nutrition of plants in these soils.

Tropical Forage Legumes

Acid soils are commonly utilized for low-input pastures. Some forage legumes are well adapted to acid conditions such

as perennial stylo (*Stylosanthes guianensis* (Aubl.) Swartz), centrosema (*Centrosema pubescens* Benth.), and tropical kudzu (*Pueraria phaseoloides* (Roxb.) Benth.) (Andrew and Vanden Berg, 1973; Spain et al., 1975; de Carvalho et al., 1981). White popinac (*Leucaena leucocephala* (Lam.) De Wit) is quite tolerant to low soil fertility and can withstand slight soil acidity, but it grows better in neutral or slightly alkaline soils (Bogdan, 1977). Furthermore, tropical forage legumes, particularly *S. humilis* and *C. pubescens*, have lower internal P requirements associated with maximum growth, compared to temperate forage legumes (Andrew and Robins, 1969; 1971). These legumes form symbiotic associations with both vesicular-arbuscular (VA) mycorrhizal fungi and *Rhizobium*.

Symbionts

Rhizobium

Rhizobium is a genus containing several species of N_2 fixing bacteria which may form symbiotic associations with legumes. The product of the symbiosis is a root nodule, an organized structure in which *Rhizobium* species can exist within plant cells. The processes whereby colonization of a legume root by *Rhizobium* takes place, leading to differentiation of the root tissue and formation of the root nodule, have been documented by Dart (1974) and Libbenga and Bogers (1974). Symbiotic N_2 fixation in the root nodule is a result of various biological and biochemical interactions

between the legume host and the *Rhizobium* symbiont. *Rhizobium* may provide, partly or completely, the N requirements of legumes through biological N_2 fixation. As much as 80-90% of the N assimilated by *Rhizobium* is imparted to the legume host (Virtanen and Miettinen, 1963). Nodulation and N_2 fixation are energy-expensive processes. Nitrogenase activity for the reduction of atmospheric dinitrogen to ammonia is dependent on ATP. Approximately 21 mol of ATP are converted to ADP per mol of N_2 reduced (Shanmugan et al., 1978). Hence, P deficiency can be a critical limiting factor for effective N_2 fixation. Azcon et al. (1988) reported that N_2 fixation rate is improved as P supply is increased. In soils where P is limiting, *Rhizobium* inoculation alone or the presence of indigenous *Rhizobium* in the soil does not guarantee successful legume establishment (Waidyanatha et al., 1979).

VA Mycorrhizal Fungi

A VA mycorrhiza is a fungus-root symbiotic association characterized by the formation of arbuscules and, in many instances, vesicles also. The hyphae of the fungal symbionts, which are members of the fungal order Glomales, penetrate the host epidermis, grow intercellulary and intracellularly in the root cortical region, and proliferate into the surrounding soil or other media. These external hyphae provide an efficient, widely distributed surface for water and nutrient absorption and can significantly increase the host's P uptake

from the soil solution (Tinker and Gildon, 1983). They remove P from beyond the nutrient depletion zone (Hattingh et al., 1973; Rhodes and Gerdemann, 1975). Polyphosphate is synthesized by the fungal symbiont from the inorganic P absorbed by the external hyphae (Capaccio and Callow, 1982) and is believed to be translocated to the arbuscules by cytoplasmic streaming (Cox et al., 1975; Callow et al., 1978; Cooper and Tinker, 1981). The arbuscules store very little of polyphosphate (Bowen et al., 1975). Instead, the latter is transferred actively to the host across living membranes (Kinden and Brown, 1975) just after a short time lag (Cooper and Tinker, 1978).

Vesicular-arbuscular mycorrhiza have improved the phosphorus nutrition, nodulation and/or N_2 fixation of *P. phaseoloides* (Waidyanatha et al., 1979; Salinas et al., 1985), *S. guianensis* (Mosse et al., 1976; Mosse, 1977; Waidyanatha et al., 1979), *C. pubescens* (Mosse et al., 1976), and *L. leucocephala* (Manjunath et al., 1984; Punj and Gupta, 1988) grown in P-deficient acid soils. These tropical forage legumes have a high mycorrhizal dependency which may be related to the degree of root hair development. Gerdemann (1975) defined mycorrhizal dependency as the degree to which a plant is dependent on the mycorrhizal condition to produce its maximum growth or yield at a given level of soil fertility. *Leucaena leucocephala* has no root hairs and is strongly mycorrhizal-dependent (Munns and Mosse, 1980; Habte

and Manjunath, 1987; Saif, 1987; Guzman-Plazola et al., 1988). *Stylosanthes guianensis* and *C. pubescens* form few root hairs and exhibit a strong dependency on mycorrhizae (Crush, 1974; Mosse, 1981; Saif, 1987).

Rationale

Vesicular-arbuscular mycorrhizal fungi can significantly increase the uptake of available P by plants, and thus may be valuable in exploiting acid mineral soils for low-input pastures. An understanding of the effects of soil acidity and Al on these fungi is necessary if they are to be utilized to improve pasture production in acid mineral soils as establishment of the symbiotic association is likely to depend on Al tolerance. Chapter III evaluates the tolerance of several isolates of VA mycorrhizal fungi to soil acidity and Al based on spore germination and hyphal growth in three acid soils with varied percent Al saturation. Tolerance may differ among genera, species, or isolates within species. Previous studies have shown that root colonization by, or effectiveness of, VA mycorrhizal fungi varies with pH and Al. It has not been shown that the difference in the ability of these fungi to colonize and improve plant growth in acid mineral soils is partly related to Al tolerance. Chapter IV evaluates the effectiveness of VA mycorrhizal fungi, which differed in tolerance to soil acidity and Al toxicity, to colonize the legumes, and improve their P nutrition, nodulation, N

nutrition, and growth in acid soils. The degree of Al tolerance of VA mycorrhizal fungi may affect their activities and overall effectiveness in acid soils.

Objectives

The overall objective of this study was to determine if VA mycorrhizal fungi selected for tolerance to soil acidity and Al at the species and isolate levels would enhance the efficiency of nodulation and N nutrition of forage legumes in a high-Al, acid soil.

The specific objectives were to: (i) isolate, characterize and identify VA mycorrhizal fungi from a high-Al acid soil, (ii) evaluate the tolerance of several VA mycorrhizal fungi to soil acidity and Al, (iii) determine if isolates of VA mycorrhizal fungi naturally sensitive to high Al saturation can develop tolerance to such factor by acclimation, and (iv) evaluate the effectiveness of several VA mycorrhizal fungi with varying degrees of tolerance to Al toxicity, in improving nutrition, nodulation, and growth of forage legumes in an acid mineral soil.

CHAPTER II
REVIEW OF LITERATURE

Nature of Acidity in Mineral Soils

Tropical soils, particularly those in the humid regions, are highly weathered. Leaching gradually removes soluble salts, minerals, and bases, and thus facilitates weathering. Iron and Al oxides, which are highly resistant to weathering, remain in the parent material. Most of such highly-weathered soils fall in the orders ultisol and oxisol (Van Wambeke, 1976). They occupy a large proportion of the tropics (Cochrane, 1978; Sanchez and Isbell, 1978), but are not confined to this region. In fact, ultisol is the most extensive soil order of southern USA (Perkins et al., 1973).

The nature of soil acidity depends on the soil chemical composition and its ion exchange and hydrolysis reactions (Thomas and Hargrove, 1984). There are two components of soil acidity, the exchangeable and the titratable, but non-exchangeable, acidity. Exchangeable acidity consists of any monomeric Al or Fe, and exchangeable H, if present. Monomeric Al^{3+} and Fe^{3+} ions, displaced from the lattices of clay minerals by cations, hydrolyze in solution. The hydrolysis of these trivalent cations contribute significantly to soil acidity (Coleman et al., 1964). The hydrolysis reactions

liberate H^+ ions and lower the soil solution pH. The H^+ ions are readsorbed and further react with soil minerals causing more hydrolysis (Ragland and Coleman, 1960; Frink and Peech, 1963; Jackson, 1963). Exchangeable acidity can be replaced, and is thus extractable, by a neutral unbuffered salt such as KCl (Lin and Coleman, 1960). Exchangeable acidity extracted from clay consists mainly of exchangeable Al^{3+} (Harward and Coleman, 1954; Low, 1955). A higher proportion of exchangeable acidity appears to be H^+, only in soils where organic matter is an important contributor to cation exchange capacity (CEC) (Yuan, 1959; 1963). Furthermore, much of the H^+ that is apparently exchangeable from organic matter arises from the hydrolysis of Al^{3+} which is difficult to replace from organic matter with KCl (Yuan, 1959). The H^+ ions produced by organic matter decomposition are unstable in mineral soils. They react with layer silicate clays, releasing exchangeable Al^{3+}. Thus, exchangeable H^+ is found only in small amounts in mineral soils.

Exchangeable acidity is the type of soil acidity that directly affects plant growth. The Al^{3+} extracted by 1N KCl gives a reasonably accurate measure of soil acidity that must be neutralized to rid of exchangeable Al^{3+} and thus, Al toxicity (Kamprath, 1970; Reeve and Sumner, 1970; Farina et al., 1980).

The other type, titratable acidity, is the amount of acid that is neutralized at a selected pH, and corresponds

approximately to acidity measured with $BaCl_2$-TEA at pH 8.2 (Pratt and Bair, 1962; Bhumbla and Mc Lean, 1965). Titratable acidity is a result of pH-dependent hydrolysis reactions involving hydroxy-Al (Coleman and Thomas, 1964), organic matter (Martin and Reeve, 1958; Schnitzer and Skinner, 1963a; 1963b), and hydrated oxides of Al and Fe (Mattson and Wiklander, 1940; Parks and de Bruyn, 1962). Hydroxy-Al is not exchangeable by neutral unbuffered salts (Rich, 1960). In organic matter, Al is present as hydroxylated Al, mostly $Al(OH)_2^+$ forming a hydroxy Al-organic matter complex, rather than as exchangeable Al (Schnitzer and Skinner, 1963a; 1963b). Aluminum in this complex is difficult to replace (Mortensen, 1963; Hargrove and Thomas, 1981a; 1981b; White and Thomas, 1981). The amount of Al adsorbed by organic matter and the degree of hydrolysis of adsorbed Al increase with an increase in pH (Hargrove and Thomas, 1981b). However, at low pH, Al in organic matter is hydrolyzed to a greater extent than Al in clays because the hydrolysis products react with the carboxyl group of organic matter more extensively than it does with clay. In addition, organic matter serves as a sink for H^+ ions produced by hydrolysis.

Effect of Acidity on Legume-*Rhizobium* Symbiosis

Rhizobium

Rhizobium and *Bradyrhizobium* species are affected by acidity, as evaluated in soil or in liquid culture. For

instance, strains of *R. meliloti* Dangeard are generally acid sensitive and their activity is restricted in soils with pH levels below 6.0 (Robson and Loneragan, 1970). Multiplication of *R. leguminosarum* (Frank) Frank biovar *trifolii* is inhibited below pH 5.0 (Wood et al., 1984a). Although all strains of *Rhizobium* and *Bradyrhizobium* usually multiply at pH 5.5 - 7.5, acid tolerance down to pH 3.5 is in the order: (*R. loti* Jarvis, Pankhurst and Patel; *B. japonicum* (Kirchner) Jordan; cowpea rhizobia) > (*R. leguminosarum* biovars *trifolii* and *phaseoli*) > *R. meliloti* (Graham and Parker, 1964; Vincent, 1977). Most species exhibit interstrain variation in terms of multiplication in the pH range 4.0 - 5.5. This has been shown in *R. leguminosarum* biovar *trifolii* (Thornton and Davey, 1983a), lotus rhizobia (Cooper, 1982), red clover rhizobia (Lindstrom and Myllyniemi, 1987), and *R. leguminosarum* biovar *phaseoli* (Karanja and Wood, 1988). A strain of *R. meliloti* which is acid tolerant in culture media persisted in a highly acid soil (Lowendorf et al., 1981). Such strain differences should be exploited in agriculture, particularly in the tropics where acid soils are widespread.

Nodulation and Nitrogen Fixation

Nodule formation in many legumes is inhibited or delayed by soil acidity (Cooper, 1989). This was observed in *Stylosanthes* species (De Carvalho et al., 1980) and other tropical legumes (Murphy et al., 1984), as well as in

temperate legumes such as white clover (*Trifolium repens* L.) (Wood et al., 1984a; 1984b). Interspecific differences exist in legumes in their abilities to nodulate and fix N_2 in acid soils. The effects of lime applications on yield and N_2 fixation of different legumes grown on a Hawaiian oxisol was evaluated by Munns et al. (1977). Cowpea (*Vigna unguiculata* (L.) Walp.), peanut (*Arachis hypogaea* L.), soybean (*Glycine max* (L.) Merill), and *Stylosanthes* species were acid tolerant, whereas, white popinac (*L. leucocephala*), crownvetch (*Coronilla varia* L.), alfalfa (*Medicago sativa* L.), and bean (*Phaseolus vulgaris* L.) were acid sensitive. Nitrogen fixation by *Stylosanthes* species was little affected by soil acidity. However, *L. leucocephala* was well nodulated at low pH but did not fix N_2 well, suggesting that its nodule function was acid sensitive. Both nodulating and N_2-fixing ability of a commercial bean inoculant, previously shown superior to the indigenous rhizobia, were markedly reduced when introduced in an acid soil (Ssali, 1981).

Legumes also have intraspecific variation in regard to nodulation and N_2-fixing abilities under acid-soil conditions. Varieties of *M. sativa* were selected for vigor in acid (pH 4.4) or limed soils (Bouton et al., 1981). The acid-selected lines had greater nodule mass and N_2 fixation at pH 4.4 than at pH 6.1, whereas, the ones selected in limed soils performed better at pH 6.1.

Effect of Aluminum on Legume-*Rhizobium* Symbiosis

Rhizobium

Sensitivity of *Rhizobium* and *Bradyrhizobium* to Al varies greatly with species and strains. Karanja and Wood (1988) screened 48 strains of *R. leguminosarum* biovar *phaseoli* isolated from Kenyan soils for tolerance to acidity and Al in liquid culture. Three strains were found tolerant to 10 μM Al at pH 4.5 but none was tolerant to 50 μM Al at a higher pH of 5.5. Rhizobia were generally more sensitive to Al than to the H^+ ion. Aluminum imposed an additional stress on multiplication of *R. leguminosarum* biovar *trifolii* other than acidity *per se* at pH 4.5 (Wood and Cooper, 1985). Similarly, at concentrations below 50 μM, Al inhibited the multiplication of *Rhizobium* which were otherwise acid tolerant (Keyser and Munns, 1979a; 1979b; Thornton and Davey, 1983a). In contrast to these findings, the H^+ ion is more important than Al in limiting the multiplication of slow-growing lotus rhizobia (*Bradyrhizobium* sp.) in liquid culture (Wood et al., 1988). Although fast- and slow-growing lotus rhizobia are equally tolerant of Al (at least 50 μM), slow-growing rhizobia are less tolerant of acidity. At pH 4.5, Al had no additional effect on multiplication of lotus rhizobia. The same was reported by Cooper (1982) that H^+ ion alone caused a decline in viability of rhizobia.

The monomeric form Al^{3+} is well-established as the most physiologically toxic form of Al (Adams and Pearson, 1967; Foy et al., 1978). However, in *R. leguminosarum* biovar *trifolii*, Al also inhibited the multiplication of the bacteria at pH 5.5 despite its polymerization and precipitation from solution (Wood and Cooper, 1984).

Aside from inhibiting multiplication of *Rhizobium* and *Bradyrhizobium*, Al affects the persistence of rhizobia in acid soils. The persistence of *R. meliloti* is limited in acid soils and has resulted in reduced nodulation of annual *Medicago* species (Robson and Loneragan, 1970).

Screening rhizobia for acid soils is based largely on the ability of *Rhizobium* strains to multiply in acid laboratory media supplied with Al (Date and Halliday, 1978; Keyser and Munns, 1979b). With this technique, strains with improved survival and nodulating ability in acid soils have been sucessfully identified in *R. leguminosarum* biovar *phaseoli* (Graham et al., 1982b; Lowendorf and Alexander, 1983), cowpea rhizobia (Keyser et al., 1979; Hartel et al., 1983) and in clover rhizobia (Thornton and Davey, 1983b), among others.

Nodulation and Nitrogen Fixation

Nodulation of temperate and tropical legumes is reduced or inhibited by Al. In *T. repens*, Al inhibited nodulation in the pH range 5.0-6.0 (Wood et al., 1984a). In *A. hypogaea*, nodulation was reduced when Al saturation of the CEC reached

30% although this level is not yet toxic to the host plant (Pieri, 1974). In Carribean stylo (*S. hamata* (L.) Taub) and shrubby stylo (*S. scabra* Vog.), Al strongly affected nodulation by delaying the appearance, and reducing the number and dry weight of nodules (De Carvalho et al., 1982b) while in *S. guianensis*, Al reduced the number of plants that became nodulated (De Carvalho et al., 1981). *Vigna unguiculata* will normally nodulate below pH 5.0. However, its nodulation is delayed or inhibited by Al even when inoculated with a *Rhizobium* strain which is acid or Al tolerant in culture media (Hohenberg and Munns, 1984).

There have been attempts to identify the stage in the nodulation process that is most affected by Al. Aluminum was suggested to interfere with root colonization by *Rhizobium* and with nodule initiation (De Carvalho et al., 1982b). In support of this, Al in nutrient solution above 0.89 mg L^{-1} inhibited the rhizobial colonization-infection process in *P. vulgaris* roots (Franco and Munns, 1982).

Nodulation is apparently more sensitive to Al toxicity than N_2 fixation. An Al concentration in nutrient solution as high as 100 μM had no detrimental effect on N_2 fixation of well nodulated *S. hamata*, Townsville stylo (*S. humilis* H.B.K.) and *S. scabra* (De Carvalho et al., 1982a). Evidently, the effect of Al on nodulation is primary and that once the legumes become nodulated, Al has little or no effect on N_2 fixation. In a study by Jarvis and Hatch (1985), Al at 50 and

100 mmol m^{-3} had considerably reduced nodulation and consequently N_2 fixation in *T. repens*. The effect of Al on nodule initiation was responsible for the marked reduction in nitrogenase activity of roots at high levels of the metal. However, even when nodule initiation was not reduced at 25 mmol m^{-3} Al^{3+}, nitrogenase activity was still adversely affected. Jarvis and Hatch speculated that Al may also interfere with the biochemical processes and mechanisms in N_2 fixation, or may reduce the supply of nutrients and metabolites to the nodules.

Effect of Acidity on VA Mycorrhizal Fungi

Spore Abundance and Distribution

Spore numbers of VA mycorrhizal fungi vary with different soil pH levels (Kruckelman, 1975; Sheikh et al., 1975). *Acaulospora laevis* Gerdemann and Trappe was the predominant VA mycorrhizal fungus in sites in Western Australia with soil pH values ranging from 4.0 to 5.6 (Abbott and Robson, 1977) with the highest number of spores at pH 4.5-4.9 (Porter, 1982; Porter et al., 1987a). When *A. laevis* was introduced into a high-pH soil, the fungus colonized fewer roots, produced fewer spores and had lower germination and shorter germ tubes than in its natural low-pH soil (Porter et al., 1987b). *Acaulospora laevis* seems tolerant of soil acidity. In fact, only *A. laevis* spores were recovered from soils in Central

Florida with pH levels ranging from 4.0 to 4.5 (Nicolson and Schenck, 1979).

Glomus mosseae (Nicolson and Gerdemann) Gerdemann and Trappe was commonly found in alkaline soils (Gerdemann and Trappe, 1974). In Western Australia, this species was not usually found in soils with pH below 5.0 (Abbott and Robson, 1977). *Glomus monosporum* Gerdemann and Trappe was more commonly found in soils with pH levels greater than 4.6 (Abbott and Robson, 1977) and the highest number of spores was found at pH 7.0-7.4 (Porter, 1982). Root colonization, sporulation, and spore germination of *Gl. monosporum* was reduced when it was introduced into a low-pH soil (Porter et al., 1987b). Soil acidity is an important factor limiting the activity and thus, the occurrence and distribution of VA mycorrhizal fungi.

Acidity may also affect the production and viability of VA mycorrhizal spores in natural soils (Read et al., 1976). However, no study to date specifically evaluated spore production by different species and isolates of VA mycorrhizal fungi in relation to pH.

Spore Germination

Vesicular-arbuscular mycorrhizal fungi have an optimum pH for spore germination, which varies with species and, probably, even with isolates. In water extract agar, *Gl. mosseae* germinated best at pH 7.0 and failed to germinate at

pH 4.0. Germination declined very rapidly with every unit decrease in pH from 7.0 (Green et al., 1976). The same observation was reported by Wong and Marschner (1988) who studied the effects of nitrogen source and concentration on spore germination and hyphal growth of *Gl. mosseae* at different pH levels. Germination was best at pH 7.0, was reduced significantly at pH 5.0, and was prevented at pH 4.0, regardless of N source or concentration. In water agar, Mosse and Hepper (1975) obtained the best germination of *Gl. mosseae* between pH 7.0 and 7.2 and germination was prevented at pH 4.9. For *Gl. versiforme* (Karsten) Berch (*Gl. epigeum* Daniels and Trappe), optimum germination in nonsterile soil occurred around pH 7.0-7.4, although germination was observed over a broad pH range from 4.8 to 8.0 (Daniels and Trappe, 1980).

Scutellispora coralloides (Trappe, Gerdemann and Ho) Walker and Sanders germinated best at pH 5.0 and germination declined rapidly with every unit increase in pH. The spores failed to germinate at pH 9.0 (Green et al., 1976). Optimum germination for *Gi. margarita* Becker and Hall (Siqueira et al., 1982) and for *S. heterogama* (Nicolson and Gerdemann) Walker and Sanders (Green et al., 1976) occurred at pH 6.0. Germination of *A. laevis* in soil was favored below pH 6.0, was optimum at pH 4.0-5.0, and was reduced in neutral or alkaline conditions (Hepper, 1984). It is now becoming apparent that

Gigaspora and *Scutellispora* species are adapted to a rather wide range of edaphic conditions including soil acidity.

Hyphal Growth

Information on the effects of acidity on hyphal growth of VA mycorrhizal fungi is scanty. Presumably, both intraradical and extraradical growth of hyphae through soils are adversely affected by extreme soil acidity. The hyphae of a *Glomus* sp. were unable to grow in acid soils unless limed to pH 5.3, while substantially more hyphae were formed at the highest pH tested (pH 7.4) (Abbott and Robson, 1985). Although the fungus had successfully penetrated red clover (*T. pratense* L.) roots at pH 5.3, the hyphae failed to spread within the roots. In another study by Siqueira et al. (1984), *Gl. mosseae* germinated in unlimed soil (pH 5.5; 68% Al saturation), but the germ tubes were stunted markedly. Liming to pH 6.1, which decreased exchangeable Al, resulted in a fourfold increase in germ tube growth. Reduced growth of germ tubes arising from VA mycorrhizal spores under acid conditions was also observed by Hepper (1979) and Hepper and Smith (1976).

It is not known how species of VA mycorrhizal fungi differ in their abilities to grow in acid soils. A VA mycorrhizal fungus which grows extensively intraradically may be less affected by soil acidity than one which grows extensively extraradically as Abbott and Robson (1985) have speculated.

Root Colonization

Glomus mosseae colonized onion (*Allium cepa* L.) well at pH 7.0 (Hayman and Mosse, 1971). Root colonization by this species was inhibited in an acid soil but was increased by liming (Siqueira et al., 1984). *Glomus macrocarpum* Tulasne and Tulasne colonized nigerseed (*Guizotia abyssinica* (L.f.) Cass.) better at pH 6.6 than at pH 4.3 to 5.6 (Graw, 1979). These observations are consistent with the reported behavior of *Glomus* species in relation to pH, particularly spore germination.

The results obtained with other *Glomus* species contradict the generalization noted above. *Glomus fasciculatum* (Thaxter) Gerdemann and Trappe emend. Walker and Koske colonized subterranean clover (*T. subterraneum* L.) extensively at soil pH 5.3-7.5 (Abbott and Robson, 1985). This species was found well established in unlimed acid soils (Mosse, 1972b). *Glomus fasciculatum* may really be adapted to acid soils, since this species was originally described from acid peat in Canada (Thaxter, 1922). Six isolates of *Gl. tenue* (Greenall) Hall differed in their ability to form VA mycorrhiza with forage legumes at low pH (Lambert and Cole, 1980). Several isolates did not colonize at low pH. However, only the fine endophyte, which Wang et al. (1985) supposed to be *Gl. tenue*, colonized the roots of spring oats (*Avena sativa* L.) at soil pH 4.5. There may be a wide intraspecific variation within *Gl. tenue* in regard to adaptability to soil pH.

Gigaspora margarita seems adapted to acid soil conditions. Siqueira et al. (1984) examined the effects of soil acidity on root colonization of corn (*Zea mays* L.) by *Gi. margarita* and *Gl. mosseae*. *Gigaspora margarita* was found much less sensitive to acid conditions than *Gl. mosseae*, although liming increased root colonization by both species. *Gigaspora gigantea* (Nicolson and Gerdemann) Gerdemann and Trappe has been found in mine spoils with pH levels lower than 4.1 (Daft et al., 1975). However, some isolates of *Gi. gigantea* failed to colonize forage plants at low pH (Lambert and Cole, 1980).

Effectiveness in Improving Plant Growth

Effectiveness of VA mycorrhizal fungi in improving P uptake and growth of the hosts varies in relation to pH. *Gigaspora margarita* increased the growth of sweetgum (*Liquidambar styraciflua* L.) in soils with pH 4.5 and 5.5, but it did not in more alkaline soils (Yawney et al., 1982). A *Glomus* sp. improved the growth and P uptake of *T. subterraneum* only when soil pH was 7.0 or higher (Abbott and Robson, 1985). *Glomus macrocarpum* improved the growth of Mexican marigold (*Tagetes minuta* L.) at pH 4.3, but had little effect at higher pH values (Graw, 1979). *Acaulospora laevis* was more effective in promoting plant growth in acid soils than in neutral soils (Mosse, 1972a; 1975).

Different VA mycorrhizal fungi have been compared for effectiveness in improving the growth of host plants over a

range of pH values. Davis et al. (1983) amended a high P-fixing soil (pH 5.0) with lime to obtain different pH levels ranging from 5.0 to 8.1. *Glomus fasciculatum* best stimulated the growth of *L. styraciflua* at low pH levels (5.1-5.9) whereas *Gl. mosseae* improved its growth only at higher pH levels (6.0-8.1).

Habte and Fox (1989) evaluated the symbiotic effectiveness of indigenous VA mycorrhizal fungi present in widely-differing tropical soils, ranging from pH 4.3 to 5.9, and compared it to the effectiveness of pot-cultured *Gl. aggregatum* Schenck and Smith emend. Koske. The indigenous VA mycorrhizal fungi in Paaloa soil (pH 4.3) produced low VA mycorrhizal colonization on *L. leucocephala* grown in limed soil (pH 6.3). When both the indigenous VA mycorrhizal fungi and *Gl. aggregatum* were used as inoculum together, there was a large growth response to inoculation because most of the colonization was produced by *Gl. aggregatum*. Apparently, the indigenous VA mycorrhizal fungi in acid soil were not effective in limed soil.

Skipper and Smith (1979) compared the effectiveness of *Gi. gigantea* and *Gl. mosseae* for *G. max* grown in an acid soil (pH 5.1), with and without lime. *Gigaspora gigantea* was effective in improving the growth of *G. max* in unlimed acid soil but *Gl. mosseae* was ineffective in unlimed soil. However, *G. max* showed large growth responses to colonization by *Gl. mosseae* after the soil has been limed to pH 6.2.

Effect of Aluminum on VA Mycorrhizal Fungi

Spore Germination and Hyphal Growth

Barkdoll (1987) studied in detail the effects of Al on different species and isolates of VA mycorrhizal fungi. Spore germination, hyphal growth, and number of penetration sites of *Gl. mosseae*, originally obtained from a soil with pH 6.6, were all negatively correlated with soil Al. Germination and penetration of *Scutellispora pellucida* (Nicolson and Schenck) Walker and Sanders isolated from an acid soil (pH 3.8), and of *Gl. manihot* Howeler, Sieverding and Schenck isolated from a high-Al, acid soil (pH 4.0; 4.8 meq Al 100 g^{-1} soil) were both correlated with soil Al. Mahmud (1983) also found spore germination of *Gl. mosseae* negatively correlated with exchangeable Al. Siqueira et al. (1984), studying spore germination and germ tube growth of *Gl. mosseae* in both soil and soil extract agar, determined Al ions were toxic to some VA mycorrhizal fungi. Obviously, the response of VA mycorrhizal fungi to Al varies with the species. *Glomus mosseae* was very sensitive, while *S. pellucida* and *Gl. manihot* proved tolerant (Barkdoll, 1987).

Root Colonization

Root colonization of *A. sativa* by *Gl. caledonium* (Nicolson and Gerdemann) Trappe and Gerdemann was severely inhibited by Al and, to some extent Mn, even at concentrations

that did not affect the growth of the host (Wang, 1984). Aluminum and Mn rather than H ions may be primarily responsible for inhibition of mycorrhizal colonization at low soil pH, since colonization was not inhibited at low pH in the absence of Al and Mn ions. In *Z. mays*, root colonization by *Gl. mosseae* was negatively correlated with Al and Zn (Siqueira et al., 1984), implicating the involvement of these metals in acid soils in inhibiting the activity of sensitive VA mycorrhizal fungi.

Gigaspora margarita may have tolerance to Al and other limiting factors in the soil-acidity complex. In an unlimed soil (pH 5.5), *Gi. margarita* colonized *Z. mays* better than did *Gl. mosseae* (Siqueira et al., 1984). Furthermore, root colonization by *Gi. margarita* was less affected by liming compared with *Gl. mosseae*. Yawney et al. (1982) also reported no significant correlations between soil pH adjustments and percent mycorrhizal roots in *L. styraciflua* colonized by *Gi. margarita*.

Vesicular-Arbuscular Mycorrhizal Fungi Isolated from Acid and/or High-Al Soils

Some VA mycorrhizal fungi were originally isolated or are known only from acid soils containing high exchangeable Al. Others have edaphotypes which are adapted to acid soils. *Acaulospora dilatata* Morton, *A. lacunosa* Morton, and *A. rugosa* Morton were described from coal mine soils and other acid, high-Al soils in West Virginia (Morton, 1986). *Acaulospora*

undulata Sieverding occurred in an acid oxisol in Zaire (Sieverding 1988). *Acaulospora appendicula* Spain, Sieverding and Schenck was found in acid soils (pH 5.0-5.5) in Florida (Schenck et al., 1984). *Acaulospora myriocarpa* Spain, Sieverding and Schenck and *A. denticulata* Sieverding and Toro were originally described from acid soils in Colombia (pH 4.5 and 5.1, respectively) (Schenck et al., 1986; Sieverding and Toro, 1987). *Acaulospora taiwania* Hu was originally isolated from an acid soil (pH 4.5) in Taiwan (Hu, 1988).

Glomus diaphanum Morton and Walker was found in acid, high-Al mine soils in West Virginia (Morton and Walker, 1984) while *Gl. callosum* Sieverding was obtained from acid oxisols in Zaire (Sieverding, 1988). *Glomus glomerulatum* Sieverding is only known from Colombia where it has been collected from an acid soil with pH 4.9 and 1.3 meq Al 100 g^{-1} soil (Sieverding, 1987). *Glomus clarum* has been isolated from acid soils in Singapore (pH 3.9) (Louis and Lim, 1988).

Scutellispora pellucida and *S. dipurpurascens* Morton and Koske were abundant in high-Al, acid (pH 4.9) minesite in West Virginia (Koske and Walker, 1986; Morton and Koske, 1988). *Scutellispora scutata* Walker and Diederichs was isolated from an oxisol in Brazil with pH 4.5, 1.45 mg kg^{-1} soil exchangeable Al, and 67% Al saturation (Walker and Diederichs, 1989). *Scutellispora biornata* Spain, Sieverding and Toro was first recovered from oxisols in the LLanos of Colombia with pH 4.8 and 0.8 meq Al 100 g^{-1} soil (Spain et al., 1989).

Effect of Other Metals on VA Mycorrhizal Fungi

Spore Germination and Hyphal Growth

Spore germination and germ tube growth of some VA mycorrhizal fungi *in vitro* were found sensitive to metals like Zn, Mn, and Cu. Addition of 0.5 mg L^{-1} Cu reduced the germination of *Gl. caledonium* spores to 16% of the control (Hepper and Smith, 1976). The toxicity of Cu to VA mycorrhizal spores was reconfirmed by Hepper (1979). While Zn toxicity inhibited germination and hyphal growth of *Gl. mosseae* (Hepper and Smith, 1976), trace amounts of Zn stimulated germination of this species in agar (McIlveen and Cole, 1979).

Root Colonization

Colonization of *P. vulgaris* roots by indigenous VA mycorrhizal fungi was reduced with addition of Zn to soil (45 and 135 $\mu g\ g^{-1}$) (McIlveen et al., 1975). Addition of Zn higher than 45 $\mu g\ g^{-1}$ soil decreased mycorrhizal colonization of *G. max* by *Gl. mosseae* while addition of small amounts enhanced root colonization (McIlveen and Cole, 1979). Manganese also inhibited root colonization of *A. sativa* by *Gl. caledonium*, but it was about ten times less inhibitory than Al at similar concentrations (Wang et al., 1985). Addition of Zn, Cu, Ni, and Cd to a soil medium strongly reduced root colonization of *A. cepa* by *Gl. mosseae* (Gildon and Tinker, 1983). The metals

Cd and Cr were more severe than Cu, Zn, Ni, or Pb in reducing total length of mycorrhizal roots formed by a *Glomus* sp. in *Z. mays* and *G. max* (Chao and Wang, 1988).

The internal Zn concentration in the root rather than Zn concentration in soil affects root colonization by VA mycorrhizal fungi. In a split-root experiment (Gildon and Tinker, 1983), *Z. mays* inoculated with *Gl. mosseae* were grown in pots with a central division. Zinc at the rate of 100 μg Zn g^{-1} soil was added on one side of the divided pot only. However, mycorrhizal colonization on both sides of the pot were reduced to the same level. Zinc translocated within the plant from the other roots to which no Zn had been added decreased mycorrhizal colonization in the whole root system.

Metal Uptake

Inoculation with *Gl. aggregatum* increased the uptake of Cu and Zn by *L. leucocephala* in an oxisol (pH raised to 6.2) (Manjunath et al., 1989). The metals accumulated in the roots of mycorrhizal plants since the Cu concentration was higher in roots than in shoots. Similar increases in Cu and Zn uptake due to VA mycorrhizal fungi were observed in *G. max* inoculated with either *Gl. mosseae* or *Gl. fasciculatum* (Pacovsky, 1986) and in *P. vulgaris* inoculated with an unidentified VA mycorrhizal fungus (Kucey and Janzen, 1987). Using radioactive isotopes, Cooper and Tinker (1978) measured the uptake and translocation of Zn by external mycelium of *Gl.*

mosseae colonizing *T. repens* roots while Swaminathan and Verma (1979) demonstrated that zinc extracted by VA mycorrhizal roots was available to the nonmycorrhizal plants, as well.

While VA mycorrhizal fungi improve plant uptake of limiting metals, they reduce plant uptake of metals which are available in toxic amounts. Iron and Mn concentrations in mycorrhizal *G. max* were lower than in equivalent non-mycorrhizal P-fertilized plants which accumulated toxic levels of Mn (Pacovsky, 1986). In other studies, mycorrhizal plants grown in high-Mn soils had taken up less Mn than their non-mycorrhizal counterparts. This was observed in sorghum (*Sorghum bicolor* (L.) Moench.) (Pacovsky et al., 1985) and *G. max* (Pacovsky et al., 1986).

Adaptation in VA Mycorrhizal Fungi

Soil pH

Vesicular-arbuscular mycorrhizal fungi have adaptation for particular soil pH ranges, as shown by Porter et al. (1987b). *Acaulospora laevis* was found the dominant VA mycorrhizal fungus in low pH soils in Western Australia while a *Glomus* sp. was dominant in high pH soils. When the pH levels of their respective soils were adjusted by liming and acidification, respectively, spore germination of *A. laevis* and of *Glomus* sp. decreased. Wang (1984) also observed adaptation for soil pH in *Gl. caledonium*. Isolates obtained from soil with pH 7.5 colonized roots less at reduced pH than

did isolates from soil at pH 5.5. In an alkaline California soil (pH 7.3), the indigenous isolates of *Gl. fasciculatum* formed significantly more external mycelia than did isolates from acid Florida soils and were more effective in enhancing growth of Troyer citrange (*Poncirus trifoliata* (L.) Raf. X *Citrus sinensis* (L.) Osbeck) (Graham et al., 1982a). *Glomus manihot* may be adapted to acid soils. This species, after introduction into an acidic oxisol with high P-fixation capacity (pH 4.4; 3.9 $\mu g\ g^{-1}$ soil P), colonized pot-grown Gamba grass (*Andropogon gayanus* Kunth.) and *P. phaseoloides* and persisted in the presence of indigenous VA mycorrhizal fungi, mainly *Entrophospora colombiana* (Salinas et al., 1985).

Phosphorus

High levels of soil P can reduce the percentage of mycorrhizal roots (Mosse, 1973; Hayman et al., 1975). However, Crush (1975) observed extensive mycorrhizal development in pasture plants growing in New Zealand soils which have been fertilized heavily for years. In Australia, Porter et al. (1978) studied the population dynamics of VA mycorrhizal fungi in a pasture soil as affected by long-term phosphate application. Separate plots received five rates of superphosphate ranging from 0 to 224 kg $ha^{-1}yr^{-1}$ in the last 10 years. Spore numbers of *A. laevis* and a *Gigaspora* sp. were not affected by superphosphate history and neither was the proportion of mycorrhizal roots in *T. subterraneum* and several

other pasture grasses. Obviously, some VA mycorrhizal fungi have become tolerant to high levels of soil P due to selection pressure created by heavy applications of superphosphate. An isolate of *Gl. dimorphicum* Boyetchko and Tewari is probably adapted to high levels of soil P. It was the only species found in an intensively managed barley fields fertilized regularly with N and P (Boyetchko and Tewari, 1986).

Phosphorus-tolerant VA mycorrhizal fungi may lose their ability to improve P uptake and plant growth in low-P soils. Louis and Lim (1988) compared two isolates of *Gl. clarum* Nicolson and Schenck obtained from soils with different levels of available P. *Glomus clarum* isolated from a low-P soil (0.03 ppm P) increased the growth of *G. max* in a P-deficient soil (0.95 ppm P). Inoculation with the isolate from a high-P soil (7.87 ppm P) did not significantly affect plant growth. In addition, in a P-deficient soil, *G. max* inoculated with a low-P isolate had greater mycorrhizal colonization, nodulation, and nitrogenase activity than those inoculated with a high-P isolate of *Gl. clarum*.

Metals

Some isolates of VA mycorrhizal fungi have likely developed an adaptation to Zn over time. The inhibitory effect of Zn on germination of these fungi differed depending on the isolate source (Hepper and Smith, 1976). Gildon and Tinker (1981) recovered *Gl. mosseae* colonizing clover plants

from a heavily Zn- and Cd-contaminated site. This was unexpected since the Rothamsted isolate of this species was found highly sensitive to Zn, Cd, Ni, and Cu in soil. Della Valle et al. (1987) isolated *Gl. versiforme* and *S. persica* (Koske and Walker) Walker and Sanders from the rhizospheres of plants growing on waste piles of a Zn mining company heavily contaminated with the metal (0.3 mg g^{-1} soil). Perhaps species of VA mycorrhizal fungi normally sensitive to metals can adapt and become exceptionally tolerant to them.

Importance of Phosphorus on Nodulation and Nitrogen Fixation

McLachlan and Norman (1961) noted increased requirement for P by nodulated legumes. Andrew and Robins (1969) demonstrated the importance of P on N nutrition of legumes. Phosphorus addition to soil increased N concentration in the shoots beyond the maximum increase in dry matter. This was attributed to improved root development and nodulation. Indeed, adequate P supply was observed important for satisfactory nodulation in *G. max* (Demetrio et al., 1972) and in *S. humilis* (Gates, 1974), among others. The level of P supply affects nodule initiation and nodule growth much more than shoot or root growth (Cassman et al., 1980). This is understandable considering that nodules contain as much as three times more P per unit dry matter than their subtending roots (Mosse et al., 1976). Nodules serve as a a sink for P. ^{32}Phosphorus foliar feeding studies proved that there is a

translocatory pathway for P from the phloem to the nodules (Moustafa et al., 1971). In *G. max*, the minimum concentration of P in the external solution required for nodulation is about 0.5 $\mu g\ L^{-1}$ (Cassman et al., 1980).

Nitrogen fixation began early in plants adequately supplied with P (Gates, 1974). Once N_2 fixation has started, P supply improved the rates as demonstrated in *M. sativa* through ^{15}N enrichment studies (Azcon et al., 1988). Moreover, P significantly increased the amount of N accretion in legumes per unit of nodule tissue (Gates, 1974). Recently, Adu-Gyamfi et al. (1989) examined the effect of P supply on absorption and utilization efficiency of P in relation to dry matter production and N_2 fixation in pigeon pea (*Cajanus cajan* (L.) Millsp.). Increasing P supply (up to 200 kg P ha^{-1}) improved N_2 fixation in all 8 cultivars tested. Since dry matter production and N_2 fixation were controlled more by P absorption ability of the plant rather than by P utilization efficiency, it was concluded that effective translocation of P to the leaf is important for N_2 fixation. The importance of P in N_2 fixation is probably related to the high requirement for ATP in N_2-fixing systems. Apparently, ATP production by aerobic pathways is essential for N_2 fixation in all legume nodules which have been studied (Bergersen, 1977; Pate, 1977). Burris (1971) speculated that increased cellular P content may enhance phosphorylation of ADP to ATP in the bacteroids. Furthermore, P is a component of carbohydrate metabolism

intermediates and thus may affect the metabolism of N_2 fixation products (Pate, 1977).

Interaction Between VA Mycorrhizal Fungi and *Rhizobium*

Competition for Infection Sites

Nodulation and VA mycorrhizal colonization proceed simultaneously, indicating that *Rhizobium* and VA mycorrhizal fungi do not compete for colonization sites (Smith and Bowen, 1979; Barea and Azcon-Aguilar, 1983). Even after colonization of roots by both symbionts, VA mycorrhizal fungi do not usually invade the nodule cortical tissues (Smith and Bowen, 1979; Mosse, 1981). In fact, VA mycorrhizal fungi have not been found in active nodules (Crush, 1974). However, Smith and Schenck (1985) observed sporulation of *Gl. ambisporum* Smith and Schenck and *Gl. heterosporum* Smith and Schenck in senescent nodules in *G. max*.

Competition for Photosynthate

Although the symbionts do not compete for root colonization sites, they compete for carbon substrate. Both *Rhizobium* and VA mycorrhizal fungi have large demands for photosynthate. This may explain why prior inoculation of one endophyte inhibited the development of the other (Bethlenfalvay et al., 1985). In one case, the highest level of VA mycorrhizal colonization by *Gl. mosseae* was obtained

without *R. japonicum* and the greatest nodule mass was obtained without a VA mycorrhizal fungus. Early introduction of *Gl. mosseae* even caused a reduction in nodule weight (Brown and Bethlenfalvay, 1986). When introduced simultaneously, VA mycorrhizal fungi may have an advantage over *Rhizobium* in periods of carbon stress. Defoliation which subjected *G. max* to carbon stress did not affect the biomass of *Glomus* but considerably reduced nodule weight and nitrogenase activity (Bayne et al., 1984), demonstrating that VA mycorrhizal fungi can develop at the expense of *Rhizobium*.

Rhizobium Favors VA Mycorrhizal Colonization

Inoculation with *Rhizobium* improved colonization of *L. leucocephala* roots by native VA mycorrhizal fungi (Manjunath et al., 1984), and colonization of chickpea (*Cicer arietinum* L.) by *Gl. fasciculatum* (Subba Rao et al., 1986). Both cell-free supernatant and intact bacterial culture of *R. meliloti* increased mycorrhizal colonization of *M. sativa* by *Glomus* sp. (Azcon et al., 1978; Azcon-Aguilar and Barea, 1978). It is not known whether *Rhizobium* favors mycorrhizal colonization by directly interacting with the external phase of the fungus or indirectly through its hormonal effects on the plant. The culture supernatant of *R. meliloti* contained plant hormones of auxin, gibberellin, and cytokinin type (Azcon et al., 1978). Auxins, for instance, affect root formation and make the cell wall more permeable (Thimann, 1972). *Rhizobium* possibly

favors VA mycorrhizal colonization through its hormonal effects on the plant.

VA Mycorrhiza Favors Nodulation and Nitrogen Fixation

Vesicular-arbuscular mycorrrhiza may have additional effects on nodulation and N_2 fixation other than that mediated by improved P nutrition. Phosphate fertilization at a rate that eliminated the mycorrhizal effect on plant growth did not eliminate mycorrhizal effect on nodulation and N_2 fixation (Asimi et al., 1980). *Glomus mosseae* stimulated nodule development and activity in *G. max* (Bethlenfalvay et al., 1987). This was associated not only with higher P levels, but also with better leaf conductance. The latter may have caused an increase in CO_2 uptake (Auge et al.,1986). The legume-*Rhizobium* symbiosis requires an additional supply of carbohydrate for nodule growth and respiration (Ryle et al., 1979). Over the whole growing period of *V. unguiculata*, 10% of the carbon from net photosynthesis was translocated to the nodules (Herridge and Pate, 1977). The requirement for carbohydrates during N_2 fixation in legumes has been calculated to be between 4 and 10 mg per mg N fixed (Pate and Herridge, 1978; Pate et al., 1979). Thus, improved leaf conductance by mycorrhiza (Allen and Boosalis, 1983; Huang et al., 1985; Koide, 1985) may favor nodulation through improved CO_2 uptake. The effect of *Gl. mosseae* on dry matter yield and nodulation of *M. sativa* by *R. meliloti* was little affected by

water potential (Azcon et al., 1988). Since P uptake in mycorrhizal plants was affected by water content, other mechanisms must be involved in mycorrhizal activity. *Phaseolus vulgaris* inoculated with *Rhizobium* and *Gl. macrocarpum* not only had more nodules and higher nitrogense activity and P contents, but also had more protein and leghemoglobin than those inoculated with *Rhizobium* only (Daft and El-Giahmi,1974). Leghemoglobin regulates the transport of O_2 from outside the nodules to the bacteroid surface (Bergersen and Goodchild, 1973). Since Co is required for the synthesis of leghemoglobin (Dilworth et al., 1979), uptake of this element is possibly improved by VA mycorrhiza. Unfortunately, no one has ever studied the effect of VA mycorrhiza on Co uptake by plants. However, VA mycorrhizal fungi have been shown to increase the plant's uptake of other trace elements (Gilmore, 1971; Munns and Mosse, 1980; Pacovsky, 1986; Manjunath et al., 1989).

Glomus mosseae stimulated nitrogenase activity in *G. max* early in plant development and preceded any growth response of the host (Asimi et al., 1980). Mycorrhizal colonization by *Gl. mosseae* and nodulation in *M. sativa* occurred simultaneously within 2 weeks after inoculation. This was also when mycorrhizal plants nodulated extensively and had higher rates of nitrogenase activity, compared to nonmycorrhizal plants (Smith and Daft, 1977). Increase in nodule dry weight, nitrogenase activity, and P content of *V.*

unguiculata inoculated with *Gl. mosseae* and *Rhizobium* started about 3 weeks after inoculation and corresponded to the onset of the development of extensive external mycelium of the fungus (Gueye et al., 1987). These findings suggest that VA mycorrhizal fungi stimulate nodulation and N_2 fixation mainly through increased uptake by extraradical hyphae of limiting nutrients. The significant increase in P uptake has long been recognized. In addition, VA mycorrhizal fungi probably improve the host's uptake of Co which is necessary for the synthesis of leghemoglobin, of Mo which is the metal component of nitrogenase, or of other trace elements which may be important for nodulation and N_2 fixation.

CHAPTER III
TOLERANCE OF SELECTED VA MYCORRHIZAL FUNGI
TO SOIL ACIDITY AND AL SATURATION

Introduction

Vesicular-arbuscular (VA) mycorrhizal fungi are known to increase the absorption of labile P by plants (Hattingh et al., 1973; Rhodes and Gerdemann, 1975; Tinker and Gildon, 1983), and thus, may be valuable in exploiting acid soils for low-input pastures. However, it is important to understand the effects of soil acidity and Al on these fungi before they can be utilized to improve pasture production in acid mineral soils where they are most needed. Effects of pH on spore germination have been examined. Half of the studies were evaluated in water agar which included *Gl. mosseae* (Mosse and Hepper, 1975; Green et al., 1976; Wong and Marschner, 1988), *S. coralloidea*, and *S. heterogama* (Green et al., 1976). The only species examined using soil were *Gl. versiforme* (Daniels and Trappe, 1980), *Gi. margarita* (Siqueira et al., 1982), and *A. laevis* (Hepper, 1984). In terms of pH effects on hyphal growth which were evaluated using soil, only *Gl. mosseae* (Siqueira et al., 1984) and an unidentified *Glomus* sp. (Abbott and Robson, 1985) have been examined. In all these studies using soil, there was no indication of an Al toxicity problem.

Since Al is more toxic to VA mycorrhizal fungi than any other predominant element in acid soil, it is important to study the effect of Al on these fungi. To date, the effect of Al on spore germination and hyphal growth of VA mycorrhizal fungi have been investigated only in *Gl. mosseae* (Mahmud, 1983; Barkdoll, 1987), *Gl. manihot*, *A. longula* and *S. pellucida* (Barkdoll, 1987). Establishment of VA mycorrhizal fungi in acid soils is likely to depend greatly on the ability of these fungi to tolerate the factors associated with strong soil acidity, particularly phytotoxic levels of Al. Tolerance may differ among genera, species, or isolates within the same species.

Barkdoll (1987) also examined the effect of Al on spore germination and hyphal growth of several species and isolates of VA mycorrhizal fungi. However, this is the only study which addressed the comparative response of these species and isolates to soil Al saturation in an attempt to relate Al tolerance with the overall effectiveness of these fungi in a high-Al, acid mineral soil.

The objectives of this study were to: (i) isolate VA mycorrhizal fungi from a soil high in acidity and Al, (ii) evaluate the tolerance of several VA mycorrhizal fungi to soil acidity and Al, and (iii) determine if isolates of VA mycorrhizal fungi naturally sensitive to high soil Al saturation can develop tolerance to such factor by acclimation.

Materials and Methods

Isolation of VA Mycorrhizal Fungi from a High-Al Acid Soil

Soil. Top soil (0-15 cm depth) of Pacolet sandy clay loam (Typic Hapludult) was collected from the University of Georgia Agricultural Experiment Station, Griffin, GA. The physical and chemical characteristics of the soil are presented in Table 3-1. The soil is acidic and contains plant-toxic levels of Al. Organic matter (%OM) was determined by Walkley-Black method (Nelson and Sommers, 1982). Soil pH was measured in H_2O (1:2 v/v). Buffer pH was determined using Adams-Evans buffer solution (Adams and Evans, 1962). The elements P, K, Ca, Mg, Mn, and Fe were extracted with Mehlich 1 extractant (0.05 N HCl in 0.025 N H_2SO_4) (Hanlon and DeVore, 1989) and analyzed in the filtrate by inductively coupled argon plasma (ICAP) spectroscopy (Soltanpour et al.,1982). Exchangeable Al, Ca, and Mg were displaced with unbuffered 1N KCl (Lin and Coleman, 1960) since the pH values of the soils were less than 5.5. The elements were analyzed in the filtrate by atomic absorption spectrometry with a N_2O-C_2H_2 flame (Willis, 1965). In addition, Al and Fe were also extracted with citrate-dithionate-bicarbonate. Percent Al saturation, the proportion of the colloidal exchange sites occupied by Al, was determined by dividing meq exchangeable Al by the effective cation exchange capacity (ECEC). The latter is the sum of exchangeable Al, Ca, and Mg.

Table 3-1. Chemical characteristics of the three acid soils.

Parameter	Pacolet sandy clay loam	Wauchula sand	Arredondo fine sand
pH	4.3	5.0	4.5
Buffer pH	7.2	7.6	7.7
1N KCl-extractable:			
Al*	157.0	30.0	14.0
Ca	trace	35.0	172.0
Mg	trace	47.0	41.0
Citrate-dithionite-bicarbonate-extractable:			
Al	1723.0	423.0	197.0
Fe	8647.0	373.0	193.0
Mehlich I-extractable:			
Ca	120.0	193.0	99.1
Mg	18.5	25.8	14.5
K	9.2	12.1	11.4
P	6.7	9.4	5.2
Mn	1.0	11.0	4.0

A composite portion of field-collected soil was saved for isolation of indigenous VA mycorrhizal fungi. The remainder, to be used for experiments, was steam-pasteurized twice at 80 C for 4 h to eliminate the indigenous VA mycorrhizal fungi (Sylvia and Schenck, 1984) and then passed through a 4-mm sieve.

Indigenous VA mycorrhizal fungi. Spores of VA mycorrhizal fungi were extracted from field-collected Pacolet sandy clay loam by wet sieving and decanting (Gerdemann and Nicolson, 1963), using sieves with apertures ranging from 45 to 425 μm. Spores were separated from organic debris and soil particles by centrifugation in H_2O, followed by centrifugation in 40% sucrose (Jenkins, 1964). The spores recovered were examined and quantified (spores g^{-1}) using a stereoscopic dissecting microscope (7X to 45X).

Spores recovered in the isolation process were used to start single-species pot cultures with Bahiagrass (*Paspalum notatum* Flugge). The grass seeds were germinated in an autoclaved sand:vermiculite mixture (1:1 v/v). Nursery cones (130-ml volume) (R. Leach Cone-Tainer Corp., Canby, OR) were filled to two-thirds with steam-pasteurized Pacolet sandy clay loam. Ten spores of indigenous VA mycorrhizal fungi were then layered on the soil in each cone. The cones were filled to the top with more soil, and two 1-month old *P. notatum* seedlings were transplanted to the tubes. The pot cultures were kept initially in a growth room equipped with mercury vapor lights

(800 $\mu mol.m^{-2}.min^{-1}$ photosynthetic photon flux density) operating for 14 h each day, and with temperatures ranging from 26 to 28 C. After a month, the pot cultures were moved to a greenhouse and maintained for another 5 months. The plants were fertilized weekly with a dilute nutrient solution containing Mo and minimal P (Table 3-2). After 6 months, the pot-culture soil containing the VA mycorrhizal propagules was either stored at 5 C, or was dried with the host plant and stored at room temperature (22-24 C).

The spores were retrieved from pot-culture soil in a manner described earlier. The spores were observed and quantified with a dissecting microscope. The spores were mounted on a glass slide in polyvinyl lactic glycerol (PVLG) (Koske and Tessier, 1983). Intact and slightly broken spores were examined with a compound microscope (100X to 1000X). The spores were characterized morphologically and identified (Schenck et al., 1984; Schenck and Perez, 1990).

Spore Germination and Hyphal Growth of VA Mycorrhizal Fungi in Acid Soils with Varying Al Saturation

Soils. Three soils with varying Al levels were selected for evaluation of tolerance of VA mycorrhizal fungi to soil acidity and Al. The top 15 cm of Pacolet sandy clay loam from the University of Georgia Agricultural Experiment Station, Griffin, GA; Wauchula sand (Ultic Haplaquods) from the Beef Research Unit, Institute of Food and Agricultural Sciences (IFAS), University of Florida, Gainesville, FL; and Arredondo

Table 3-2. Composition of nutrient solution supplied to plants grown in Pacolet sandy clay loam.

Reagent	Compound Concentration mg/L	Element Concentration mg/L
$Ca(H_2PO_4)_2 \cdot H_2O$	3.15	0.77 P
		0.50 Ca
K_2SO_4	43.60	19.56 K
$CaSO_4 \cdot 2H_2O$	86.00	20.00 Ca
$MgSO_4$	15.04	3.00 Mg
H_2MoO_4	0.02	0.01 Mo

fine sand (Grossarenic Paleudult) from the Division of Plant Industry, Agriculture Food and Consumer Services, Department of Agriculture, Gainesville, FL, were collected. The soils were steam-pasteurized to eliminate propagules of indigenous VA mycorrhizal fungi. After pasteurization, soil analyses were done following the methods described earlier. The physical and chemical properties of the soils are presented in Table 3-1.

VA mycorrhizal fungi. *Glomus manihot* isolated from Pacolet sandy clay loam (INVAM Isolate LMNH 980) and other isolates of VA mycorrhizal fungi from INVAM were evaluated for tolerance to soil acidity and Al. Tolerance was based on spore germination and hyphal growth of the fungi in three soils. The species, INVAM isolate number, and origin of the VA mycorrhizal fungi are listed in Table 3-3. These VA mycorrhizal fungi were grown in pot cultures of *P. notatum*.

Spore germination and hyphal growth assay. The spores of VA mycorrhizal fungi were collected from pot cultures by wet sieving and decanting, and sucrose centrifugation as outlined earlier. The spores on the sieves were washed with deionized water until free of sucrose and were then backwashed into a 15-cm diameter Petri dish. The spores were observed directly at 7X to 45X magnification using a dissecting microscope. Mature spores, free of visible surface contaminants and other obvious defects, were picked up individually with an Eppendorf pipette and transferred into a dish of deionized water.

Table 3-3. International Culture Collection of VA Mycorrhizal fungi (INVAM) isolate number and origin of selected VA mycorrhizal fungi used in the study.

Species	INVAM Isolate	Origin	pH of Original Soil
Acaulospora appendicula Spain, Sieverding, and Schenck	AAPD 130	Florida	
Acaulospora spinosa Walker and Trappe	ASPN 257	Florida	
	ASPN 629	Colombia	
Acaulospora longula Spain and Schenck	ALGL 316	Colombia	
	ALGL 652	Colombia	
Acaulospora scrobiculata Trappe	ASBC 456	Brazil	
Entrophospora colombiana Spain and Schenck	ECLB 356	Unknown	
Entrophospora schenckii Sieverding and Toro	ESHK 383	Colombia	
Glomus mosseae	LMSS 156	Florida	
(Nicolson and Gerdemann)	LMSS 313	Georgia	
Gerdemann and Trappe	LMSS 378	Colombia	7.6
Glomus etunicatum	LETC 236	Florida	
Becker and Gerdemann	LETC 329	Georgia	5.8
	LETC 455	Brazil	
Glomus clarum Nicolson and Schenck	LCLR 551	Colombia	
Glomus manihot Howeler, Sieverding, and Schenck	LMNH 980	Georgia	4.3
Scutellispora calospora	CCLS 269	North Dakota	
(Nicolson and Gerdemann)	CCLS 348	New York	6.6
Walker and Sanders			
Scutellispora pellucida (Nicolson and Schenck) Walker and Sanders	CPLC 288	Colombia	
Scutellispora heterogama (Nicolson and Gerdemann) Walker and Sanders	CHTG 139	Unknown	
Gigaspora margarita	GMRG 185	Florida	
Becker and Hall	GMRG 444	Brazil	
Gigaspora gigantea	GGGT 109	Unknown	
(Nicolson and Gerdemann)	GGGT 663	West Virginia	
Gerdemann and Trappe			

Spores were passed through a series of such transfers until free of soil particles, organic debris, and detached hyphae. In *Glomus* species, most spore germination occurs by regrowth of the subtending hyphae, thus, their subtending hyphae were cut to a length equivalent to the diameter of the spore. Thirty to forty spores of each isolate were sandwiched between two 25-mm diameter millipore filters (Gelman Sciences Inc., Ann Arbor, MI). The filters with spores were then placed in a tissue specimen bag (Shandon Southern Instruments, Inc., Sewickley, PA) and buried in moistened test soils contained in covered sterilizing trays (Fisher Scientific, Inc., Orlando, FL). The moisture contents of the soils during the germination assay were maintained at approximately field capacity (14% moisture equivalent to -116 mbar for Pacolet sandy clay loam; 8% and -82 mbar for Wauchula sand; and 13% and -58 mbar for Arredondo fine sand). The assay was done in three replicates for each isolate in every soil and was repeated once. The experiment with *Gi. gigantea* was not repeated due to unavailability of spores free of hyperparasites. The millipore filters were retrieved from the soil after 21 d incubation in the dark at room temperature (22-24 C), and gently cleaned of adhering soil particles by a camel-hair brush and a fine stream of water. The spores and hyphae between the millipore filters were stained with a few drops of 0.05% aqueous trypan blue. Spore germination and hyphal growth were evaluated by direct microscopic examination

with a dissecting or a compound microscope (100X to 400X). Spore germination was expressed as a percentage of the total number of spores examined. Spores which produced germ tubes greater than their diameter were considered to have germinated. Hyphal growth was estimated by line-hypha intersect method modified from Newman (1966), a technique widely used for estimating root and hyphal lengths. A 25-mm diameter plastic sheet, with parallel lines 1 mm apart, was laid over the millipore filters which contain the hyphal growth. The number of line-hypha intersects were counted, and transformed to hyphal length. The mycelial growth index produced from a certain population of spores, e.g., 100 spores, was calculated from percent spore germination and hyphal length per germinated spore.

Statistical analyses. General Linear Models (GLM) was performed on the data. Data expressed as percentage were submitted to arcsine transformation (Little and Hills, 1978) prior to analyses. Since the experiments were repeated, trial 1 and trial 2 data were merged and subjected to repeated-measures analysis of variance (repeated MANOVA). There was a significant interaction between trial and VA mycorrhizal fungi for the dependent variables, spore germination, hyphal length, and mycelial growth index. Thus, the data from the two trials were presented separately. Furthermore, as the interaction between soil percent Al saturation and VA mycorrhizal fungi was significant, comparison of means due to Al saturation was

done for each fungal isolate and that due to VA mycorrhizal fungi was done at each Al saturation level. Significant difference among treatment means was determined by Waller-Duncan K-ratio T test while significant difference between genera was determined by orthogonal contrasts. Statistical Analysis Systems (SAS Institute Inc., 1986; 1988) was used in all analyses.

Acclimation of VA Mycorrhizal Fungi to Soil Acidity and Al

Soil. Steam-pasteurized Pacolet sandy clay loam was diluted with sand to obtain varying levels of soil Al saturation. Coarse (2 mm) quartz sand (The Feldspar EPK Sand Corp., Edgar, FL) was washed several times with deionized water and autoclaved at 135 C and 15 psi for 1 h. Pacolet sandy clay loam was mixed with sand at 1:7, 1:3, and 1:1 (v/v) to obtain 12.5%, 25%, and 50% Pacolet soil, respectively.

VA mycorrhizal fungi. Isolates of VA mycorrhizal fungi which did not germinate in Pacolet sandy clay loam and thus had no tolerance to high Al level were used in this study. These included *Gl. mosseae* LMSS 156, LMSS 313, LMSS 378, *Gl. etunicatum* LETC 236, LETC 329, LETC 455, *A. appendicula* AAPD 130, *A. spinosa* ASPN 257, ASPN 629, *A. longula* ALGL 316, *E. colombiana* ECLB 356, and *E. schenckii* ESHK 383.

Acclimation. The VA mycorrhizal fungi were acclimatized to soil acidity and high Al by culturing them on P. notatum in soils with progressively increasing soil acidity and percent

soil Al saturation, starting at 12.5% Pacolet sandy clay loam, relative to sand. The pot cultures were prepared as described previously, maintained in a walk-in growth room, and fertilized three times a week with a dilute nutrient solution (Table 3-2). The pot cultures were harvested after 4 months, and the spores produced were recovered. Part of the spores recovered was saved for evaluation of tolerance, as described below, while another part was used to start similar pot cultures in 25% Pacolet sandy clay loam. By repeating this process, the spores produced were progressively transferred to 50% Pacolet sandy clay loam, after every 4 months and evaluated for tolerance.

Evaluation of tolerance. After growth at each level of soil-sand mixture, spores produced were evaluated for tolerance to high Al. Tolerance was evaluated in terms of spore germination and hyphal growth in unamended Pacolet sandy clay loam, and was compared to those of unconditioned spores. The same methods for set-up and assay of spore germination and hyphal growth, as described in the preceding study, were followed.

Results

Isolation of a VA Mycorrhizal Fungus from a High-Al Acid Soil

Glomus manihot was the only species of indigenous VA mycorrhizal fungi found predominant in Pacolet sandy clay

loam. It was retrieved from field-collected soil and successfully put into single-species pot culture. This species was present in the field soil at 1 spore 25 g^{-1} soil, which is extremely low. In pot culture, it reached as high as 50 spores g^{-1} soil. This isolate has been deposited in the International Culture Collection of VA Mycorrhizal Fungi (INVAM) as *Gl. manihot* LMNH 980.

Spore Germination and Hyphal Growth of VA Mycorrhizal Fungi in Acid Soils with Varying Al Saturation

There was a significant ($p \leq 0.01$) interaction between soil percent Al saturation and VA mycorrhizal fungi affecting both germination and hyphal growth of the latter. Thus, the effect of Al saturation on VA mycorrhizal fungi is presented and interpreted separately for each isolate. Likewise, the differences in spore germination and hyphal growth due to VA mycorrhizal fungi is presented and interpreted within a particular soil or Al saturation level.

All species of *Gigaspora* except *Gi. gigantea* GGGT 663 had high tolerance to soil acidity and Al (Figures 3-1 and 3-2). The two isolates of *Gi. margarita* responded differently. While the spore germination of *Gi. margarita* GMRG 185 was increased, that of GMRG 444 was not affected as Al saturation increased from 12% to 100%. The latter isolate had lower germination in Wauchula sand than in the other soils. Likewise, the two isolates of *Gi. gigantea* behaved differently. The germination of *Gi. gigantea* GGGT 109 was not

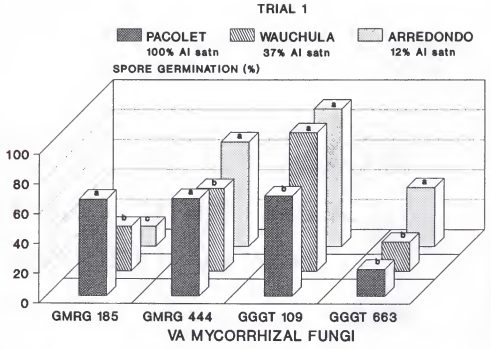

Figure 3-1. Spore germination of *Gigaspora* species in three acid soils with varying percent Al saturation (Trial 1). Means represent 3 replicates. Within an isolate, means with the same letter are not significantly different at $p \leq 0.05$ by Waller-Duncan K-ratio T test. GMRG= *Gi. margarita*, GGGT= *Gi. gigantea*.

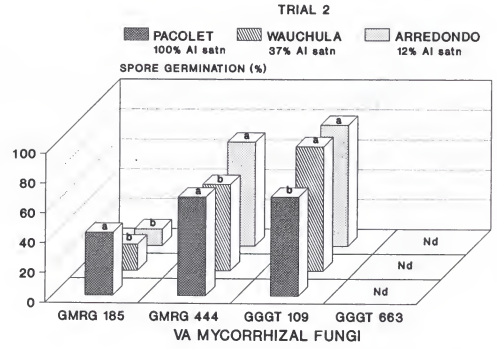

Figure 3-2. Spore germination of *Gigaspora* species in three acid soils with varying percent Al saturation (Trial 2). Means represent 3 replicates. Within an isolate, means with the same letter are not significantly different at $p \leq 0.05$ by Waller-Duncan K-ratio T test. GMRG= *Gi. margarita*, GGGT= *Gi. gigantea*, Nd= Not determined.

reduced until at 100% Al saturation while GGGT 663 was reduced at a lower level of Al equivalent to 37% saturation.

The growth of *Gi. margarita* GMRG 185 was reduced in one of two trials but that of GMRG 444 was consistently unaffected by increasing Al in the exchange site (Figures 3-3 and 3-4). *Gigaspora gigantea* GGGT 109 showed preference for greater Al whereby its hyphal growth was stimulated as percent Al saturation increased from 12% all the way up to 100%. The other isolate *Gi. gigantea* GGGT 663 was not affected by Al.

The spore germination of *most Scutellispora* species was not affected by Al within the range tested (Figures 3-5 and 3-6). *Scutellispora heterogama* CHTG 139, *S. pellucida* CPLC 288, and *S. calospora* CCLS 348 germinated equally well in all three Al-saturated soils. The germination of the latter was lower in Wauchula sand than in Arredondo fine sand in one of two trials. *Scutellispora calospora* CCLS 269 behaved differently. Its spore germination decreased as Al saturation increased from 37% to 100%.

Aluminum affected the growth of *Scutellispora* species except *S. pellucida* CCLS 269 (Figures 3-7 and 3-8). Hyphal growth of *S. heterogama* CHTG 139 and *S. calospora* CCLS 348 was not affected up to 37% Al saturation but was reduced significantly as Al saturation further increased to 100%. *Scutellispora pellucida* CPLC 288 was less tolerant than *S. heterogama* CHTG 139; its hyphal growth was reduced as Al

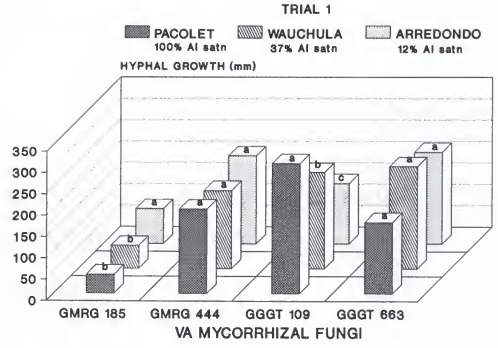

Figure 3-3. Hyphal growth of *Gigaspora* species in three acid soils with varying percent Al saturation (Trial 1). Means represent 3 replicates. Within an isolate, means with the same letter are not significantly different at $p \leq 0.05$ by Waller-Duncan K-ratio T test. GMRG= *Gi. margarita*, GGGT= *Gi. gigantea*.

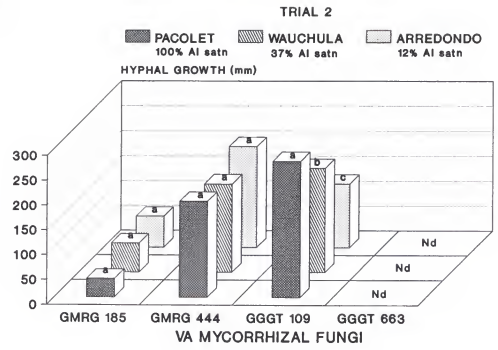

Figure 3-4. Hyphal growth of *Gigaspora* species in three acid soils with varying percent Al saturation (Trial 2). Means represent 3 replicates. Within an isolate, means with the same letter are not significantly different at $p \leq 0.05$ by Waller-Duncan K-ratio T test. GMRG= *Gi. margarita*, GGGT= *Gi. gigantea*.

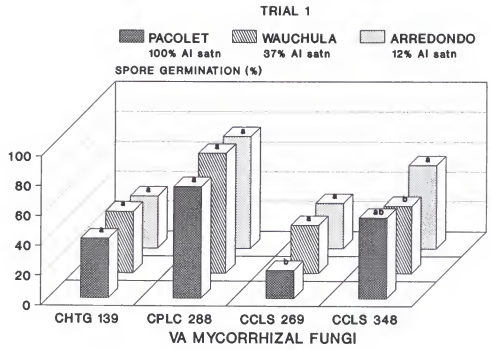

Figure 3-5. Spore germination of *Scutellispora* species in three acid soils with varying percent Al saturation (Trial 1). Means represent 3 replicates. Within an isolate, means with the same letter are not significantly different at $p \leq 0.05$ by Waller-Duncan K-ratio T test. CHTG= *S. heterogama*, CPLC= *S. pellucida*, CCLS= *S. calospora*.

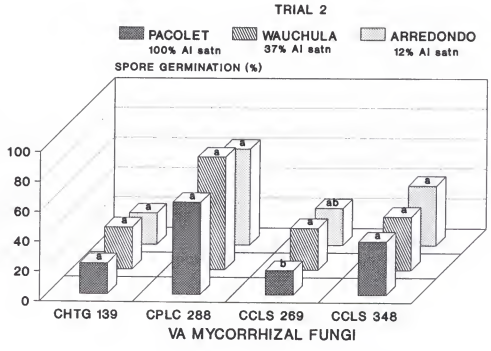

Figure 3-6. Spore germination of *Scutellispora* species in three acid soils with varying percent Al saturation (Trial 2). Means represent 3 replicates. Within an isolate, means with the same letter are not significantly different at $p \leq 0.05$ by Waller-Duncan K-ratio T test. CHTG= *S. heterogama*, CPLC= *S. pellucida*, CCLS= *S. calospora*.

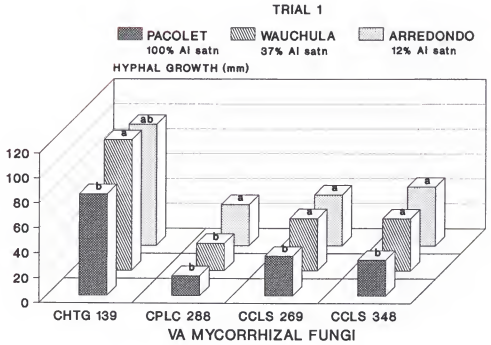

Figure 3-7. Hyphal growth of *Scutellispora* species in three acid soils with varying percent Al saturation (Trial 1). Means represent 3 replicates. Within an isolate, means with the same letter are not significantly different at $p \leq 0.05$ by Waller-Duncan K-ratio T test. CHTG= *S. heterogama*, CPLC= *S. pellucida*, CCLS= *S. calospora*.

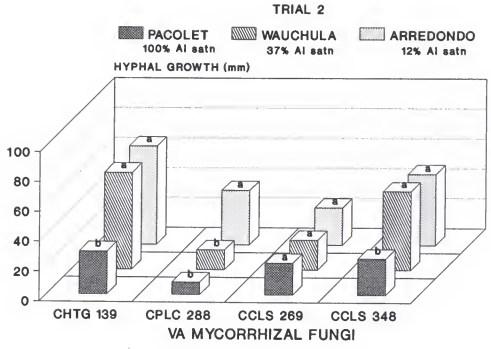

Figure 3-8. Hyphal growth of *Scutellispora* species in three acid soils with varying percent Al saturation (Trial 2). Means represent 3 replicates. Within an isolate, means with the same letter are not significantly different at $p \leq 0.05$ by Waller-Duncan K-ratio T test. CHTG= *S. heterogama*, CPLC= *S. pellucida*, CCLS= *S. calospora*.

saturation increased from 12% to 37%. Isolate differences were noted for *S. calospora* CCLS 269 and CCLS 348.

Glomus manihot LMNH 980 was the only species in the genus which consistently had high germination in all the Al-saturated soils (Figures 3-9 and 3-10). The three isolates of *Gl. etunicatum* LETC 236, LETC 329, and LETC 455 failed to germinate in Pacolet sandy clay loam but germinated well in Arredondo fine sand. Germination of these isolates was reduced starting at 37% Al saturation. Like *Gl. etunicatum* LETC 455, *Gl. clarum* LCLR 551 was adversely affected by every increment of Al. In contrast to *Gl. etunicatum,* few spores of *Gl. clarum* LCLR 551 germinated in Pacolet soil. Spore germination of *A. scrobiculata* ASCB 456 in Wauchula sand was lower than in the other two soils.

The growth of *Gl. manihot* LMNH 980 was not affected up to 37% Al saturation but was reduced as Al saturation was further increased to 100% (Figures 3-11 and 3-12). Hyphal growth of all species of *Gl. etunicatum* LETC 236, LETC 329, and LETC 455 as well as *Gl. clarum* LCLR 551 was reduced starting at 37% Al saturation. *Acaulospora scrobiculata* consistently produced very short hyphae in all test soils.

Percent spore germination, hyphal length per germinated spore, and mycelial growth index of the different isolates of VA mycorrhizal fungi in Pacolet sandy clay loam were compared with each other in order to screen those which can tolerate the highest Al level. Among all VA mycorrhizal fungi

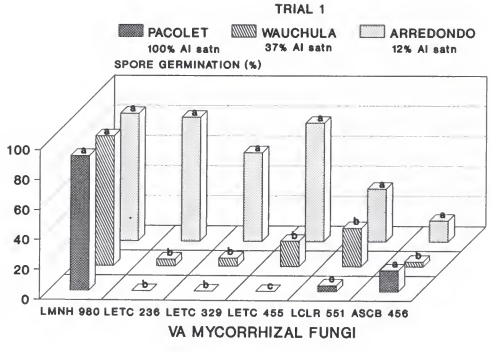

Figure 3-9. Spore germination of *Glomus* species and *A. scrobiculata* in three acid soils with varying percent Al saturation (Trial 1). Means represent 3 replicates. Within an isolate, means with the same letter are not significantly different at $P \leq 0.05$ by Waller-Duncan K-ratio T test. LMNH= *Gl. manihot*, LETC= *Gl. etunicatum*, LCLR= *Gl. clarum*, ASCB= *A. scrobiculata*.

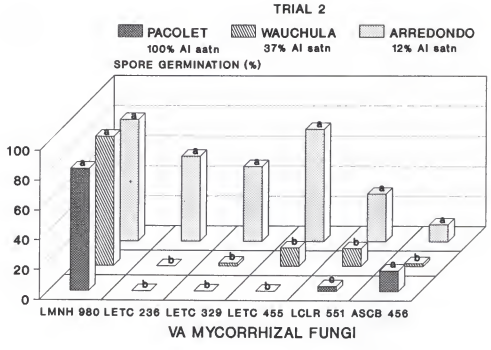

Figure 3-10. Spore germination of *Glomus* species and *A. scrobiculata* in three acid soils with varying percent Al saturation (Trial 2). Means represent 3 replicates. Within an isolate, means with the same letter are not significantly different at $p \leq 0.05$ by Waller-Duncan K-ratio T test. LMNH= *Gl. manihot*, LETC= *Gl. etunicatum*, LCLR= *Gl. clarum*, ASCB= *A. scrobiculata*.

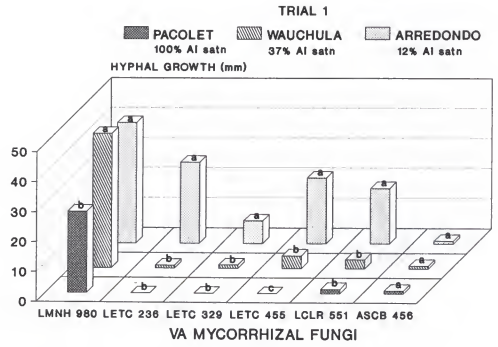

Figure 3-11. Hyphal growth of *Glomus* species and *A. scrobiculata* in three acid soils with varying percent Al saturation (Trial 1). Means represent 3 replicates. Within an isolate, means with the same letter are not significantly different at $p \leq 0.05$ by Waller-Duncan K-ratio T test. LMNH= *Gl. manihot*, LETC= *Gl. etunicatum*, LCLR= *Gl. clarum*, ASCB= *A. scrobiculata*.

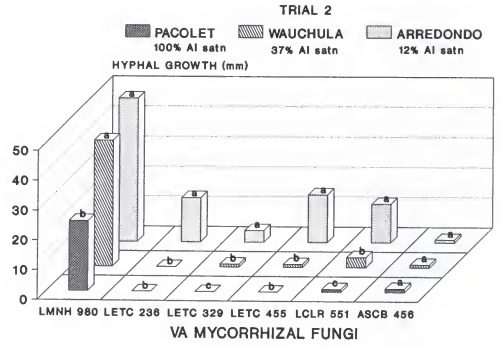

Figure 3-12. Hyphal growth of *Glomus* species and A. *scrobiculata* in three acid soils with varying percent Al saturation (Trial 2). Means represent 3 replicates. Within an isolate, means with the same letter are not significantly different at $p \leq 0.05$ by Waller-Duncan K-ratio T test. LMNH= *Gl. manihot*, LETC= *Gl. etunicatum*, LCLR= *Gl. clarum*, ASCB= *A. scrobiculata*.

evaluated, *Gl. manihot* LMNH 980 had the highest germination which was significantly different from any other isolate (Figures 3-13 and 3-14). *Gigaspora* and *Scutellispora* species also demonstrated high tolerance to Al. Next to *Gl. manihot* LMNH 980, they had the highest percent spore germination in Pacolet sandy clay loam. The performance of *Gigaspora margarita* GMRG 185 and GMRG 444, *Gi. gigantea* GGGT 109, and *S. pellucida* CPLC 288 were not different from one another, but was better than that of *S. calospora* CCLS 348, and *S. heterogama* CHTG 139. *Gigaspora gigantea* GGGT 663 and *S. calospora* CCLS 269 had lower spore germination than the other members of these genera. Differences in spore germination among isolates of the same species were detected. *Gigaspora gigantea* GGGT 109 had higher spore germination than GGGT 663, and *S. calospora* CCLS 348 had higher germination than CCLS 269. Except for *Gl. manihot* LMNH 980, *Glomus* species were generally found sensitive to soil acidity and Al. All three isolates of *Gl. etunicatum*, LETC 236, LETC 329, and LETC 455 failed to germinate in Pacolet sandy clay loam. *Glomus clarum* LCLR 551 germinated but the value was too low to be significant. *Acaulospora scrobiculata* ASCB 456 had low germination comparable to that of *S. calospora* CCLS 269.

In regard to hyphal length per germinated spore (Figures 3-15 and 3-16), *Gl. manihot* LMNH 980 produced shorter hyphae than most *Gigaspora* and *Scutellispora* species. *Gigaspora gigantea* GGGT 109 grew most extensively, followed by *Gi.*

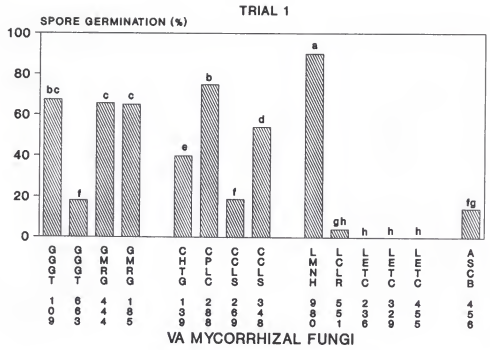

Figure 3-13. Spore germination of selected VA mycorrhizal fungi in 100% Al-saturated Pacolet sandy clay loam (Trial 1). Means represent 3 replicates. Means with the same letter are not significantly different at p≤0.05 by Waller-Duncan K-ratio T test. GGGT= *Gigaspora gigantea*, GMRG= *Gi. margarita*, CHTG= *Scutellispora heterogama*, CPLC= *S. pellucida*, CCLS= *S. calospora*, LMNH= *Glomus manihot*, LCLR= *Gl. clarum*, LETC= *Gl. etunicatum*, ASCB= *Acaulospora scrobiculata*.

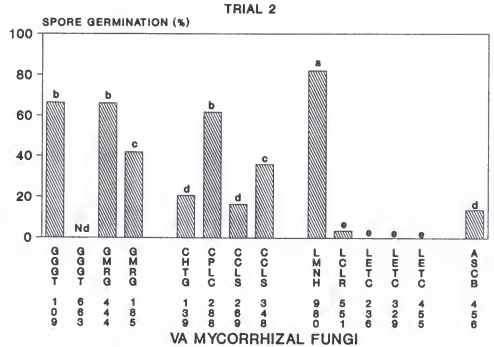

Figure 3-14. Spore germination of selected VA mycorrhizal fungi in 100% Al-saturated Pacolet sandy clay loam (Trial 2). Means represent 3 replicates. Means with the same letter are not significantly different at $p \leq 0.05$ by Waller-Duncan K-ratio T test. GGGT= *Gigaspora gigantea*, GMRG= *Gi. margarita*, CHTG= *Scutellispora heterogama*, CPLC= *S. pellucida*, CCLS= *S. calospora*, LMNH= *Glomus manihot*, LCLR= *Gl. clarum*, LETC= *Gl. etunicatum*, ASCB= *Acaulospora scrobiculata*, Nd= Not determined.

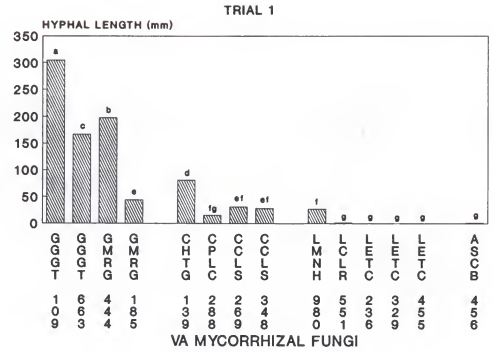

Figure 3-15. Hyphal growth of selected VA mycorrhizal fungi in 100% Al-saturated Pacolet sandy clay loam (Trial 1). Means represent 3 replicates. Means with the same letter are not significantly different at p≤0.05 by Waller-Duncan K-ratio T test. GGGT= *Gigaspora gigantea*, GMRG= *Gi. margarita*, CHTG= *Scutellispora heterogama*, CPLC= *S. pellucida*, CCLS= *S. calospora*, LMNH= *Glomus manihot*, LCLR= *Gl. clarum*, LETC= *Gl. etunicatum*, ASCB= *Acaulospora scrobiculata*.

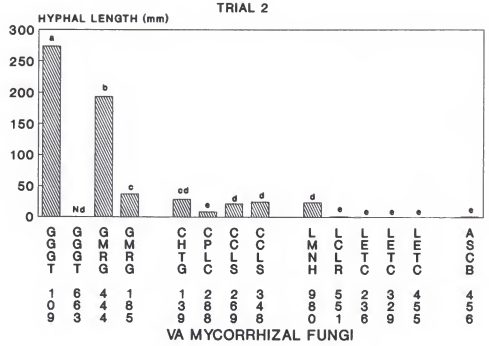

Figure 3-16. Hyphal growth of selected VA mycorrhizal fungi in 100% Al-saturated Paclolet sandy clay loam (Trial 2). Means represent 3 replicates. Means with the same letter are not significantly different at p≤0.05 by Waller-Duncan K-ratio T test. GGGT= *Gigaspora gigantea*, GMRG= *Gi. margarita*, CHTG= *Scutellispora heterogama*, CPLC= *S. pellucida*, CCLS= *S. calospora*, LMNH= *Glomus manihot*, LCLR= *Gl. clarum*, LETC= *Gl. etunicatum*, ASCB= *Acaulospora scrobiculata*, Nd= Not determined.

margarita GMRG 444, *Gi. gigantea* GGGT 663, and *S. heterogama* CHTG 139. *Gigaspora margarita* GMRG 185 had less hyphae than GMRG 444. Isolates of *Glomus*, other than *Gl. manihot* LMNH 980, had poor or no growth at all in Pacolet sandy clay loam and Wauchula sand.

The greatest mycelial growth index was obtained from *Gi. gigantea* GGGT 109 and *Gi. margarita* GMRG 444; the former was significantly better than the latter (Figure 3-17). *Gigaspora margarita* GMRG 185, *Gi. gigantea* GGGT 663, *S. heterogama* CHTG 139, *S. calospora* CCLS 348, and *Gl. manihot* LMNH 980 did not differ from one another. The least mycelia was obtained from *S. pellucida* CPLC 288 and *S. calospora* CCLS 269 whose growth was not different from that of *S. calospora* CCLS 348 and *Gl. manihot* LMNH 980. The growth from *Gl. clarum* LCLR 551 and *A. scrobiculata* ASBC 456 was negligible. Comparing isolates of the same species, *Gi. margarita* GMRG 444 was better than GMRG 185; *Gi. gigantea* GGGT 109 was higher than GGGT 663; and *S. calospora* CCLS 348 did not differ from CCLS 269. For some isolates, a similar relationship was observed when the experiment was repeated (Figure 3-18). However, *Gl. manihot* LMNH 980 was better than *S. heterogama* CHTG 139, *S. pellucida* CPLC 288, and *S. calospora* CCLS 269 in trial 2.

In general, *Gigaspora* species performed better in Pacolet sandy clay loam than *Scutellispora* species which, in turn, performed better than *Glomus* and *Acaulospora* species (Table 3-4).

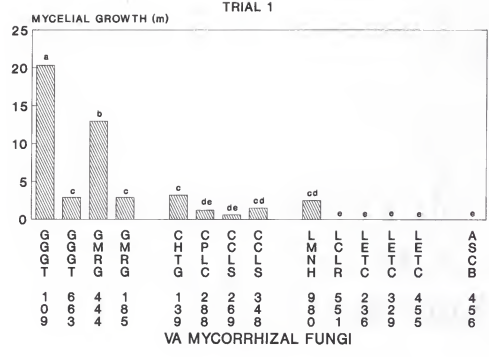

Figure 3-17. Mycelial growth index of selected VA mycorrhizal fungi in 100% Al-saturated Pacolet sandy clay loam (Trial 1). The values were calculated for a population of 100 spores considering percent spore germination and hyphal growth per germinated spore. Means represent 3 replicates. Means with the same letter are not significantly different at $p \leq 0.05$ by Waller-Duncan K-ratio T test. GGGT= *Gigaspora gigantea*, GMRG= *Gi. margarita*, CHTG= *Scutellispora heterogama*, CPLC= *S. pellucida*, CCLS= *S. calospora*, LMNH= *Glomus manihot*, LCLR= *Gl. clarum*, LETC= *Gl. etunicatum*, ASCB= *Acaulospora scrobiculata*.

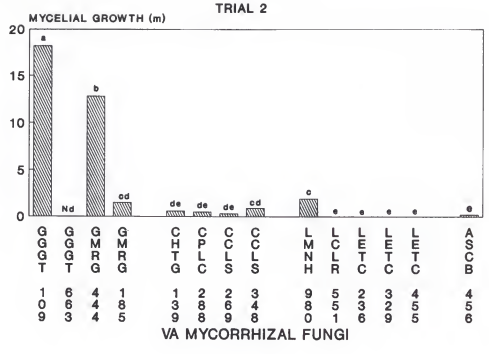

Figure 3-18. Mycelial growth index of selected VA mycorrhizal fungi in 100% Al-saturated Pacolet sandy clay loam (Trial 2). The values were calculated for a population of 100 spores considering percent spore germination and hyphal growth per germinated spore. Means represent 3 replicates. Means with the same letter are not significantly different at p≤0.05 by Waller-Duncan K-ratio T test. GGGT= *Gigaspora gigantea*, GMRG= *Gi. margarita*, CHTG= *Scutellispora heterogama*, CPLC= *S. pellucida*, CCLS= *S. calospora*, LMNH= *Glomus manihot*, LCLR= *Gl. clarum*, LETC= *Gl. etunicatum*, ASCB= *Acaulospora scrobiculata*, Nd= Not determined.

Table 3-4. Orthogonal contrasts between genera of VA mycorrhizal fungi for spore germination, hyphal growth, and mycelial growth index (MGI) in a 100% Al-saturated soil.

	Spore Germination	Hyphal Length	MGI
Trial 1:			
Gigaspora VS *Scutellispora*	*	**	**
Gigaspora VS *Glomus*	**	**	**
Gigaspora VS *Acaulospora*	**	**	**
Scutellispora VS *Glomus*	**	**	*
Glomus VS *Acaulospora*	ns	ns	ns
Trial 2:			
Gigaspora VS *Scutellispora*	**	**	**
Gigaspora VS *Glomus*	**	**	**
Gigaspora VS *Acaulospora*	**	**	**
Scutellispora VS *Glomus*	**	**	ns
Glomus VS *Acaulospora*	ns	ns	ns

ns= not significant at $p \leq 0.05$; *= significant at $p \leq 0.05$; **= significant at $p \leq 0.01$.

Acclimation of VA Mycorrhizal Fungi to Soil Acidity and Al

For most isolates of VA mycorrhizal fungi studied, there was no indication of acclimation to high Al after culturing them in increasing concentration of Pacolet soil (Table 3-5). None of the isolates cultured in 12.5% Pacolet soil was able to germinate in the tolerance assay. However, after passage in 25% Pacolet soil, spore germination was observed in *Gl. etunicatum* LETC 329, *A. longula* ALGL 316, and *Entrophospora* ECLB 356. Furthermore, *Gl. mosseae* LMSS 313 germinated after culturing in 50% Pacolet soil. For most isolates, the maximum germination obtained after acclimation was low. *Glomus etunicatum* LETC 329 developed tolerance to Al earlier than *Gl. mosseae* LMSS 313, but it had lower percent germination and more extensive hyphal growth than the latter. Further subculturing of *Gl. etunicatum* LETC 329 in 50% Pacolet soil improved its hyphal growth. The frequency of developing tolerance to Al was low. In *Glomus*, germination in 100% Al-saturated Pacolet soil after acclimation was obtained only in one of three isolates of *Gl. mosseae* and of *Gl. etunicatum*. Moreover, only one among four isolates of *Acaulospora* and one of two isolates of *Entrophospora* developed tolerance. Further subculturing in 50% Pacolet soil did not improve the performance of *A. longula* ALGL 316 but did increase the germination of *E. colombiana* ECLB 356. The latter attained

Table 3-5. Maximum spore germination (SG) and hyphal length (HL) of VA mycorrhizal fungi in 100% Al-saturated Pacolet sandy clay loam after acclimation in 12.5%, 25%, and 50% of the same soil.

		0	12.5%	25%		50%	
Species	Isolate	SG %	SG %	SG %	HG μ	SG %	HG μ
Gl. mosseae	LMSS 156	0	0	0	0	0	0
	LMSS 313	0	0	0	0	13.6	1350
	LMSS 378	0	0	0	0	0	0
Gl. etunicatum	LETC 236	0	0	0	0	0	0
	LETC 329	0	0	02.5	2000	03.6	4000
	LETC 455	0	0	0	0	0	0
A. appendicula	AAPD 130	0	0	0	0	0	0
A. spinosa	ASPN 257	0	0	0	0	0	0
	ASPN 629	0	0	0	0	0	0
A. longula	ALGL 316	0	0	20.8	3200	19.4	2900
E. colombiana	ECLB 356	0	0	27.1	16300	34.0	8100
E. schenckii	ESHK 383	0	0	0	0	0	0

* Statistical analysis was not performed owing to the nature of the data.

Table 3-5. Maximum spore germination (SG) and hyphal length (HL) of VA mycorrhizal fungi in 100% Al-saturated Pacolet sandy clay loam after acclimation in 12.5%, 25%, and 50% of the same soil.

Species	Isolate	0	12.5%	25%		50%	
		SG %	SG %	SG %	HG μ	SG %	HG μ
Gl. mosseae	LMSS 156	0	0	0	0	0	0
	LMSS 313	0	0	0	0	13.6	1350
	LMSS 378	0	0	0	0	0	0
Gl. etunicatum	LETC 236	0	0	0	0	0	0
	LETC 329	0	0	02.5	2000	03.6	4000
	LETC 455	0	0	0	0	0	0
A. appendicula	AAPD 130	0	0	0	0	0	0
A. spinosa	ASPN 257	0	0	0	0	0	0
	ASPN 629	0	0	0	0	0	0
A. longula	ALGL 316	0	0	20.8	3200	19.4	2900
E. colombiana	ECLB 356	0	0	27.1	16300	34.0	8100
E. schenckii	ESHK 383	0	0	0	0	0	0

* Statistical analysis was not performed owing to the nature of the data.

the highest germination and the most extensive growth in pure Pacolet soil among the isolates evaluated.

Discussion

To have found *Gl. manihot* as the only species of VA mycorrhizal fungi predominant in Pacolet sandy clay loam, an extremely acidic, highly Al-saturated soil, was not expected. *Glomus* species are believed to be very sensitive to acid conditions. The optimum pH range for *Gl. mosseae* is about pH 7.0-7.2 (Mosse and Hepper, 1975; Green et al., 1976; Hepper and Smith, 1976). Thus, this species is commonly found in alkaline soils (Gerdemann and Trappe, 1974). *Glomus versiforme* (*Gl. epigeum*) has an optimum range of about pH 7.0 -7.4 (Daniels and Trappe, 1980). *Glomus monosporum* is more commonly found in soils with pH levels greater than 4.6 (Abbott and Robson, 1977) and the highest number of spores was found at pH 7.0-7.4 (Porter, 1982). The predominance of *Gl. manihot* LMNH 980 in Pacolet soil suggests that tolerance to soil acidity and Al varies with species within a genus, or that a genus which is generally sensitive to soil acidity and Al may have members with the ability to adapt to these conditions. In fact, other *Glomus* species were found recently in acid soils. *Glomus diaphanum* was found in acid, high-Al mine spoils in West Virginia (Morton and Walker, 1984) and *Gl. callosum* in acid oxisols in Zaire (Sieverding, 1988). Moreover, *Gl. glomerulatum* was isolated from an acid soil in

Colombia with pH 4.9 and 1.3 meq Al 100 g^{-1} soil (Sieverding, 1987) and *Gl. clarum* from an acid soil in Singapore with pH 3.9 (Louis and Lim, 1988).

In the present study on the response of VA mycorrhizal fungi to increasing soil percent Al saturation, the moisture content of the soils used was maintained at approximately field capacity (within a narrow range of matric potential) where maximum germination of VA mycorrhizal fungi is usually obtained (Daniels and Trappe, 1980; Koske, 1981; Sylvia and Schenck, 1983). With near-equal matric potentials, the difference in texture of the soils used in the study should not become a confounding factor in studying the effect of Al saturation on VA mycorrhizal fungi. Adebayo and Harris (1971) found no apparent effect of soil texture on growth of *Phytophthora cinnamomi* and *Alternaria tenuis* at a given matric potential. The moisture contents at field capacity of the three soils used in this study were near equal despite the difference in soil texture due to the low specific surface area of low-activity (non-swelling) clay in Pacolet sandy clay loam. Skempton (1953) defined clay activity as the ratio of the plasticity index and percent clay. Pacolet soil has low cation exchange capacity and is predominated by aluminum oxide. Soils with low-activity clays are generally better aggregated than those with high-activity clays (Uehara and Gillman, 1981). Aluminum and iron oxides contribute to aggregation by forming bonds between clay particles (Deshpande

et al., 1964). The tendency of these soils to form water-stable aggregates contribute to their low water-holding capacity (Lepsch and Buol, 1974) and high water intake rate. Since pore size between aggregates increases as aggregate size increases, many highly weathered soils of the tropics have moisture release curves similar to sandy soils (Sharma and Uehara, 1968a; 1968b). Like sandy soils, strongly aggregated soils attain field capacities at comparatively low tensions, at a soil matric potential of about -100 mbar (Uehara and Gillman, 1981).

The effect of the interaction between soil percent Al saturation and VA mycorrhizal fungi on spore germination and hyphal growth of the latter was highly significant. This implies that the performance of VA mycorrhizal fungi in different soils with varying levels of Al saturation depended on the particular species or isolate involved. Spore germination of some isolates was reduced in highly Al-saturated soils while others were not affected at all. Likewise, the performance of different VA mycorrhizal fungi relative to each other depended on soil Al saturation.

Spores and hyphae are the major infecting propagules of VA mycorrhizal fungi. The former is considered the long-term survival structure (Bolan and Abbott, 1983). Spore germination and subsequent growth of hyphae through soil probably determine the rapidity of initial colonization and extent of root colonization (Abbott and Robson, 1982) which in

turn determine partly the effectiveness of VA mycorrhizal fungi. Spore germination and hyphal growth are the initial activities of VA mycorrhizal fungi which would likely be most sensitive to Al and soil acidity (Siqueira et al., 1984). In this study, both parameters were often affected by Al in a similar manner. However, there were few instances where one was reduced by Al while the other was not or was even increased. A similar phenomenon was reported by Siqueira (1983) in regard to the effect of N on spore germination and hyphal growth of VA mycorrhizal fungi. Moreover, Siqueira et al. (1982) found that germ tube growth of *Gi. margarita* was less affected by pH than was germination.

Soil pH affects spore germination (Daniels and Trappe, 1980; Siqueira et al., 1982; Hepper, 1984) and hyphal growth (Siqueira et al., 1984; Abbott and Robson, 1985) of VA mycorrhizal fungi. However, soil pH is not a solitary but a unified factor. Changes in soil pH are associated with changes in the concentration and activity of various elements in the soil. It is not known whether the observed effects of soil pH on VA mycorrhizal fungi is due to H^+, Al^{+++}, or other elements which vary with pH. In plants, the pH must be less than 3.0 before the H^+ itself becomes toxic (Bohn et al., 1979). Between pH 4.1 and pH 8.0, H^+ concentration does not limit the growth of most crops (Foy, 1974; Moore, 1974). In the present study, the pH of the soils used ranged from 4.3 to 5.0 and did not vary in the same manner as percent Al

saturation. The results clearly showed that Al^{+++}, which is the true acidity, rather than H^{+} affected VA mycorrhizal fungi. The factor to which VA mycorrhizal fungi respond to is not pH *per se*. This probably explains why Hayman and Tavares (1985) found that some VA mycorrhizal fungi did not always show the same optimum pH in different soils. Although there is an optimal pH range for spore germination of VA mycorrhizal fungi, this may vary with soil type.

Aluminum was detrimental to spore germination and subsequent hyphal growth of VA mycorrhizal fungi. This confirms the results of Barkdoll (1987) who has done a pioneering study on this aspect. This element has also been shown detrimental to activities of other fungi such as spore germination of *Neurospora tetrasperma* (Ko and Hora, 1972) and mycelial growth of *Aphanomyces euteiches* (Lewis, 1973), *Verticillium albo-atrum* (Orellana et al., 1975), and *Phytophthora capcisi* (Muchovej et al., 1980). Aluminum may have influenced the activities of VA mycorrhizal fungi by interfering with cell division and cell wall deposition, reducing DNA replication, and increasing cell wall rigidity (McCormick and Borden, 1974; Klimashevskii et al., 1979; Hecht-Buchholz and Foy, 1981). The onset of germination in *Gigaspora* species is marked by increased activity and redistribution of spore cytoplasm followed by nuclear division, thickening of innermost spore wall layer, and formation of a germ tube initial (Sward, 1981a). The growth

of germ tube wall results from *de novo* synthesis and deposition of new wall material (Sward, 1981b). Similarly, formation of new wall layers has been described by Mosse (1970) for germination of *A. laevis*. Aluminum may have affected one or more of these biochemical processes associated with spore germination in VA mycorrhizal fungi. The effect of Al on DNA replication may be important. Ethidium bromide, which specifically inhibits the synthesis of mitochondrial DNA, inhibited spore germination and germ tube growth of VA mycorrhizal fungi (Hepper, 1979; Beilby, 1983).

Manganese is the only element which may have been a confounding factor in evaluating the effect of acidity and Al on VA mycorrhizal fungi in the present study. However, this is not likely since Mn concentration was actually least in Pacolet soil, the most Al-saturated. Moreover, the effect of Mn in inhibiting root colonization of oats by *Gl. caledonicum* was five times less than that of Al (Wang, 1984). Other heavy metals such as Cd, Ni, Cu, and Pb may be present in toxic quantities only in mine spoils and not in natural acid mineral soils.

The response of VA mycorrhizal fungi to increasing soil acidity and Al varied with genera, species, and isolates of the fungi as Barkdoll (1987) concluded. Difference in response among isolates within a species of VA mycorrhizal fungi to heavy metals has been reported previously (Gildon and Tinker, 1981; 1983; Haas and Krikum, 1985). The present study

showed that tolerance is generally in the order: *Gigaspora* > *Scutellispora* > *Glomus*. This should be interpreted with caution as every isolate of VA mycorrhizal fungi is different. A generalization cannot be made for *Acaulospora* because of the problem with spore dormancy (Tommerup, 1983).

Most species of *Gigaspora* showed high tolerance to Al stress. Often, spore germination was not affected by Al saturation as in *Gi. margarita* GMRG 444 or was even increased as in GMRG 185. Similarly, hyphal growth of *Gi. margarita* GMRG 444 and *Gi. gigantea* GGGT 663 was not affected, while that of GGGT 109 was increased by increasing Al saturation. Aluminum did not affect the spore germination of most *Scutellispora* species but did reduce their hyphal growth. *Glomus* species proved extremely sensitive to Al except *Gl. manihot* LMNH 980 which showed consistently high germination in all the Al-saturated soils.

The mechanism for tolerance of some VA mycorrhizal fungi to Al is not known and is beyond the scope of the present study. The Al^{+++} is amphoteric and is exchangeable with other cations and anions. Differences in chemical composition of spores and hyphae as well as in physiology of the spore wall may regulate Al^{+++} adsorption and uptake and, thus, may be important in Al tolerance in this group of fungi. In Ascomycetes, the dormant ascospores of *N. tetrasperma* are impermeable to Al^{+++} (Lowry et al., 1957).

In the present study, the spore germination of *Gl. manihot* LMNH 980 was much higher than that of *Gl. mosseae*. On the contrary, Barkdoll (1987) found *Gl. mosseae* had higher germination than *Gl. manihot* at 0.70 meq Al. However, it would be erroneous to interpret that *Gl. mosseae* is more tolerant than *Gl. manihot* at this Al level. The discrepancy in the results was due to the manner by which germination was evaluated which in *Glomus* species would be indicated by a new growth of the subtending hypha. On this basis, *Gl. mosseae* germinated more than *Gl. manihot*, but the germ tubes of the former were soon aborted because of Al toxicity. Such a problem was avoided in the present study where new growth of the subtending hyphae was not considered as germination until its length reached twice the diameter of the spore. To score whether the spore did or did not germinate was straightforward since the subtending hyphae of *Glomus* spores for the germination assay were previously cut to a length similar to the diameter of the spore.

Hyphal growth by itself may not be a good criterion for comparing isolates encompassing different genera because of the confounding effect of energy reserve or what Bowen (1987) referred to as "driving force". In this study, the primary source of energy for growth of VA mycorrhizal fungi through soil must have been spore reserves since the assay was done without a living root. Spores have very high concentrations of lipids which could serve as energy source (Beilby and

Kidby, 1980). This energy reserve would probably depend on spore size. The final size of infection units vary with species or isolates (Abbott and Robson, 1978). Obviously, *Gigaspora* species with large spores filled with oil globules may have an advantage over *Glomus* species with relatively smaller spores. Thus, *Gl. manihot* LMNH 980 produced consistently shorter hyphae than most *Gigaspora* and *Scutellispora* species.

Spore germination alone can be used as a criterion for screening VA mycorrhizal fungi for Al tolerance. Barkdoll (1987) suggested root penetration points which she found was more sensitive to Al than was germination. However, an evaluation of this parameter is laborious and time consuming. Moreover, she did not demonstrate whether or not there was a relationship between the number of penetration points and root VA mycorrhizal colonization. Chapter IV shows a good correlation between spore germination and mycorrhizal colonization. The latter was correlated with tissue P content and host growth response, the ultimate functions of the mycorrhizal symbiosis. Screening for Al tolerance was done in soil as ecological studies done in agar or solution culture often have little or no relevance to what is observed in soil (Bowen, 1980). Although a soil environment was provided in the present study, the hyphae were allowed to grow on membrane filters. As such, hyphal growth was along a plane surface and there were no physical, chemical, and environmental

microgradients that occur naturally across pores. Nevertheless, linear length of hyphae which is important in considering nutrient uptake (Bowen, 1987) was shown in this study to be affected by soil Al saturation.

It would have been ideal if spore germination and hyphal growth were also evaluated in Pacolet soil which has been limed to pH 5.5 to rid it of exchangeable Al. This soil should have served as a control to demonstrate the relative performance of different isolates of VA mycorrhizal fungi in the absence of Al and would have reflected the inherent differences among isolates.

Aluminum tolerance may be an important factor in the selection of VA mycorrhizal fungi adapted to many acid mineral soils. Future research should be directed towards understanding the effect of Al on the biochemical processes in spore germination and elucidating the mechanisms of Al tolerance in VA mycorrhizal fungi. Furthermore, it is interesting to determine if these fungi can be induced to adapt to high-Al soils.

The study demonstrated that development of tolerance to high soil Al is possible by acclimation. The observed frequency of this phenomenon among isolates of VA mycorrhizal fungi was low, within the time frame of the study and the range of percent Al saturation where they were acclimatized. Probably, further acclimation at higher levels of Al saturation and much longer time will enable other isolates to

develop tolerance to Al, as well. A similar kind of acclimation may have occurred in isolates of *Gl. mosseae* (Gildon and Tinker, 1981), *Gl. versiforme* and *S. persica* (Della Valle et al. (1987) that were recovered in Zn-contaminated sites.

The factors involved in the observed development of tolerance to Al by some isolates of VA mycorrhizal fungi after acclimation may be genetic or environmental. If it is genetic, development of tolerance may occur by mutation or by gene amplification. It is probably more difficult to obtain an Al-tolerant phenotype by single mutation owing to the multinucleate nature of these fungi. For instance, *G. gigantea* and *G. erythropa* have 2,600 to 3,850 nuclei (Cooke et al., 1987). The frequency of spontaneous mutations is generally in the order of $1x10^{-6}$. If mutation occurs in VA mycorrhizal fungi at about this frequency, the expression of the mutants conferring Al tolerance will be masked by the remaining thousands of nonmutant nuclei. Accumulation of such mutations will be required before any observable tolerance to high levels of Al is obtained. On the other hand, VA mycorrhizal fungi, being multinucleate, increase their chance of developing tolerance by gene amplification. If the gene for Al tolerance is present in a single copy, it may be amplified to multiple copies. Amplification of hygromycin drug resistance gene was reported recently in *Fusarium* (Powell and Kistler, 1990). This gene is amplified free of the

chromosome. If the mechanism involved is nongenetic, the change induced by selection pressure may only be temporary. An isolate which has developed tolerance to high Al by acclimation may lose its tolerance if maintained in a soil without exchangeable Al. Neither genetic nor epigenetic factors can be ruled out while awaiting more studies on this aspect. In either case, the development of Al tolerance will probably be slow and only a small percentage of the population will likely develop tolerance. Nevertheless, this phenomenon is of great significance in extending the ecological sites of normally Al-sensitive VA mycorrhizal fungi.

CHAPTER IV
EFFECT OF SEVERAL VA MYCORRHIZAL FUNGI VARYING IN TOLERANCE TO SOIL ACIDITY AND AL ON NODULATION AND NUTRITION OF FORAGE LEGUMES IN A HIGH-AL ACID SOIL

Introduction

Acid mineral soils, often limited by Al toxicity coupled with N and P deficiencies, are commonly exploited for low-input pastures. Even then, these soils need a considerable amount of N and P fertilizer to support normal growth of forage legumes adapted to acid conditions. *Rhizobium* and VA mycorrhizal fungi can be utilized to reduce N and P fertilization in these soils. However, *Rhizobium* alone does not always improve legume growth because of P deficiency (Bartolome, 1983). Nodulation and N_2 fixation have high requirements for P. The level of P supply affects nodule initiation and growth (Cassman et al., 1980), the onset (Gates, 1974), and rate of N_2 fixation (Azcon et al., 1988; Adu-Gyamfi et al., 1989). Thus, VA mycorrhizal fungi, which can enhance P uptake by plants by increasing the absorbing area of roots, have improved nodulation and/or N_2 fixation of *P. phaseoloides* (Waidyanatha et al., 1979), *S. guianensis* (Mosse, 1977), *L. leucocephala* (Manjunath et al., 1984; Punj

and Gupta, 1988) and *C. pubescens* (Mosse et al., 1976) in P-deficient and/or acid soils.

Previous studies have shown that root colonization by *Gl. mosseae* (Siqueira et al., 1984) and *Gl. macrocarpum* (Graw, 1979) was inhibited in an acid soil, but not that of *Gl. fasciculatum* (Abbott and Robson, 1985). Moreover, isolates of *Gl. tenue* were found to differ in the ability to form VA mycorrhiza at low pH (Lambert and Cole, 1980). The effectiveness of VA mycorrhizal fungi in improving P uptake and plant growth likewise varies in relation to pH as demonstrated in *Gi. margarita* (Yawney et al., 1982), *Gl. macrocarpum* (Graw, 1979), *A. laevis* (Mosse, 1975), and *Gl. fasciculatum* (Davis et al., 1983). There has been no report on the effect of Al on effectiveness of VA mycorrhizal fungi, but there were on root colonization by *Gl. caledonicum* (Wang, 1984) and *Gl. mosseae* (Siqueira et al., 1984). It has not been shown, although it has been inferred, that the difference in ability of VA mycorrhizal fungi to colonize and improve host growth in acid soils is related to the degree of Al tolerance of these fungi. The results presented in Chapter III show that there are interspecific and sometimes, intraspecific variations in Al tolerance of VA mycorrhizal fungi. The degree of Al tolerance may affect the formation of mycorrhiza, extent of mycorrhizal colonization, effectiveness in enhancing P uptake, and consequently, effectiveness in

improving nodulation, N nutrition, and growth of forage legumes in an acid soil.

The objective of this study was to evaluate the effectiveness of several VA mycorrhizal fungi with varying degree of tolerance to soil acidity and Al levels, in improving the nutrient status, nodulation and growth of forage legumes in a high-Al acid soil.

Materials and Methods

Forage Legumes and Soil

Four tropical forage legumes, tropical kudzu (*Pueraria phaseoloides* (Roxb.) Benth.), perennial stylo (*Stylosanthes guianensis* (Aubl.) Swartz), white popinac (*Leucaena leucocephala* (Lam.) De Wit), and centrosema (*Centrosema pubescens* Benth.) were used as test crops. A high percentage of hard seeds in these legumes necessitated seed treatment to obtain reasonable germination. The seeds were immersed in hot water (80 C) for 1 to 10 min, and then soaked in water (24 C) for 12 to 24 h prior to sowing. Treated seeds were germinated in autoclaved vermiculite:sand mixture (1:1 v/v). At 7 to 10 d after emergence, seedlings of uniform size were selected for the experiments. The test soil was Pacolet sandy clay loam selected for its high Al saturation and soil acidity (Table 3-1). Sieved, steam-pasteurized soil was mixed with autoclaved quartz sand feldspar at 3:1 (v/v) soil:sand.

VA Mycorrhizal Fungi and *Rhizobium*

Isolates of VA mycorrhizal fungi which were found to have different levels of tolerance to soil acidity and Al in earlier studies (Chapter III), were evaluated for their effects on growth, nodulation, and nutrient content of forage legumes grown in Pacolet soil. The VA mycorrhizal fungi selected were *Acaulospora scrobiculata* (INVAM Isolate ASCB 456), *Entrophospora colombiana* (ECLB 356), *Glomus mosseae* (LMSS 378), *Gl. etunicatum* (LETC 236), *Gl. manihot* (LMNH 980), *Scutellispora calospora* (CCLS 269 and CCLS 348), *S. pellucida* (CPLC 288), *S. heterogama* (CHTG 139), *Gigaspora margarita* (GMRG 185 and GMRG 444), and *Gi. gigantea* (GGGT 109 and GGGT 663). These fungi were grown in pot cultures with *P. notatum* for about 6 months. The spores were collected from pot culture material by wet sieving and decanting, followed by sucrose centrifugation. Mature spores, free of visible defects or contaminants, were selected for inoculation. Spores were layered onto the potting medium at 40 to 50 spores per cone-tainer.

Commercially available *Rhizobium* inoculant powder (Nitragin Company, Milwaukee, WI) was used. *Pueraria phaseoloides* and *C. pubescens* were inoculated with *Rhizobium* "Type EL", *S. guianensis* with "Type Stylosanthes Spec. 4", and *L. leucocephala* with "Type L". The seedlings were inoculated with *Rhizobium* by dipping their roots in the appropriate

inoculant powder prior to transplanting. Each seedling root received about 3 to 5 mg of the inoculant.

Growth Room Experiments

Separate experiments were set up for each of the four forage legumes. The pots were prepared as described previously. Two plants were maintained in each cone for *S. guianensis* and *L. leucocephala* and one plant for *P. phaseoloides* and *C. pubescens*. The experiments on *P. phaseoloides* and *S. guianensis* contained 15 to 17 treatments consisting of 11 to 13 isolates of VA mycorrhizal fungi with *Rhizobium*, a VA mycorrhizal fungus (*Gl. manihot* LMNH 980) without *Rhizobium*, *Rhizobium* alone, and a noninoculated control. The isolates evaluated and treatment replication in *P. phaseoloides* and *S. guianensis* experiments are summarized in Table 4-1. The experiments on *L. leucocephala* and *C. pubescens* contained 4 treatments, with *Gl. manihot* LMNH 980 being the only VA mycorrhizal fungus tested. Treatments were replicated 24 times in *L. leucocephala* experiment and 12 times in *C. pubescens*. All nonmycorrhizal plants received 2 ml of spore washing filtered through a 45-μm sieve, and further through Whatman 1 filter paper. The plants were arranged in a walk-in growth room described previously, following a completely randomized design (CRD), and were rearranged every two weeks.

Table 4-1. Isolates of VA mycorrhizal fungi evaluated and treatment replication in *Pueraria phaseoloides* and *Stylosanthes guianensis* experiments.

	P. phaseoloides		*S. guianensis*	
VAM Isolates	Trial 1	Trial 2	Trial 1	Trial 2
ASCB 456	+	+	+	+
ECLB 356	+	+	+	+
LMSS 378	+	+	+	+
LETC 236	+	+	+	+
LETC 329	+	-	+	+
LETC 455	+	-	-	-
LMNH 980	+	+	+	+
CCLS 269	-	+	-	-
CCLS 348	+	+	+	+
CPLC 288	+	+	+	+
CHTG 139	+	+	+	+
GMRG 185	+	+	+	-
GMRG 444	+	+	+	+
GGGT 109	+	+	+	+
GGGT 663	-	+	-	-
Treatments	17	16	16	15
Replication	9-12	4-8	12-18	6-10

The plants were fertilized three times a week with a dilute nutrient solution (Table 3-2). To control mites, the plants were sprayed with Safer™ insecticidal soap (SAFER Inc., Wellesley, MA) every 2 months. *Pueraria phaseoloides* (Trial 1: Jul-Nov 1989; Trial 2: Mar-Jul 1989) and *C. pubescens* (Sep 1989-Jan 1990) were harvested after 4 months, while *S. guianensis* (Trial 1: Aug 1989-Feb 1990; Trial 2: Apr-Oct 1989) and *L. leucocephala* (Sep 1989-Mar 1990) were harvested after 6 months. The experiments with *P. phaseoloides* and *S. guianensis* were repeated. Results of a repeated experiment are presented as Trial 1 and Trial 2.

Measurements

Root colonization by VA mycorrhizal fungi. About 0.25 g fresh root sampled from each pot was cleared in 10% KOH and stained in aqueous trypan blue (Phillips and Hayman, 1970). Roots of *S. guianensis* and *L. leucocephala* were cleared further in alkaline H_2O_2, and stained with trypan blue in acidic glycerol (Koske and Gemma, 1989). The percentage of root colonization by VA mycorrhizal fungi was estimated visually at 7X to 45X magnification by gridline-intersect method (Giovanetti and Mosse, 1980). Visual evaluation of root colonization was facilitated using transmitted light in a dissecting microscope.

Growth and nodulation. Fresh and dry (60 C, 12 h) weights of shoots and roots, as well as, the number and dry

weight of nodules were determined. Plant height, root collar diameter, shoot length, number of leaves, and number of internodes were obtained in one or more host legumes.

Nutrient analyses. Shoot and root dried tissues were ground to pass through 1-mm mesh. Nitrogen and P concentrations in the tissues were determined at the Forage Evaluation Support Laboratory, University of Florida, Gainesville, FL. The samples were digested using a modification of the aluminum block digestion procedure of Gallaher et al. (1975). Digestion was conducted with H_2SO_4 and H_2O_2 for 4 h at 400 C. Ammonia in the digestate was determined by semiautomated colorimetry (Hambleton, 1977). Nitrogen and P concentrations were reported as a percentage of the dry matter (DM). The latter was determined by redrying the previously dried sample for 15 h at 105 C. Total N and P contents were calculated from nutrient concentration and tissue dry weight.

Statistical analyses. General Linear Models was performed on the data. Percent root mycorrhizal colonization values were submitted to arcsine transformation prior to analysis. Significant differences among treatments were determined by Waller-Duncan K-ratio T test. Correlations among the measured variables were analyzed by Pearson product-moment and Spearman rank correlation. Statistical Analysis Systems (SAS Institute Inc., 1986; 1988) was used in all analyses.

Results

Pueraria phaseoloides (Trial 1)

Root VA mycorrhizal colonization. Establishment of a VA mycorrhizal association with *P. phaseoloides* grown in high-Al, acid Pacolet sandy clay loam depended on the species of mycorrhizal fungi (Table 4-2). Seedlings inoculated with *Gl. manihot* LMNH 980, *Gi. margarita* GMRG 185 and 444, *Gi. gigantea* GGGT 109, *A. scrobiculata* ASCB 456, *S. heterogama* CHTG 139, *S. calospora* CCLS 348, and *S. pellucida* CPLC 288 were sucessfully colonized, but not those inoculated with *Gl. etunicatum* LETC 236, LETC 329, and LETC 455, *Gl. mosseae* LMSS 378, and *E. colombiana* ECLB 356. Moreover, the extent of mycorrhizal colonization differed among species of the fungi, as well as among isolates within a species. *Glomus manihot* LMNH 980 colonized the host most extensively. Colonization by *Gi. margarita* GMRG 185, GMRG 444, *Gi. gigantea* GGGT 109 and *A. scrobiculata* ASCB 456 was greater than colonization by *S. heterogama* CHTG 139, *S. calospora* CCLS 348, and *S. pellucida* CPLC 288. Comparing isolates of the same species, *Gigaspora margarita* GMRG 444 colonized the legume more thoroughly than GMRG 185. *Rhizobium* stimulated root colonization by *Gl. manihot* LMNH 980 by a magnitude of 87%. Seedlings not inoculated with VA mycorrhizal fungi remained nonmycorrhizal throughout the duration of the growthroom experiment.

Table 4-2. Root VA mycorrhizal colonization of *Pueraria phaseoloides* inoculated with selected VA mycorrhizal fungi and *Rhizobium* (Trial 1).

Treatment	Root VAM colonization %
VAMF + *Rhizobium*:	
Gl. manihot LMNH 980	78.2*a**
Gi. margarita GMRG 444	61.8 b
Gi. gigantea GGGT 109	54.5 c
A. scrobiculata ASCB 456	46.5 e
Gi. margarita GMRG 185	56.0 c
S. heterogama CHTG 139	50.6 d
S. calospora CCLS 348	41.8 f
S. pellucida CPLC 288	41.0 f
E. colombiana ECLB 356	0.0 g
Gl. etunicatum LETC 236	0.0 g
Gl. etunicatum LETC 329	0.0 g
Gl. etunicatum LETC 455	0.0 g
Gl. mosseae LMSS 378	0.0 g
Control:	
Gl. manihot LMNH 980	41.8 f
Rhizobium	0.0 g
Noninoculated	0.0 g

* Means represent 9 to 12 replicates.

** Means with the same letter are not significantly different at $p \leq 0.05$ by Waller-Duncan K-ratio T test.

Phosphorus nutrition. For this experiment and the succeeding ones, nutrition and nodulation data for plants inoculated with Al-sensitive VA mycorrhizal fungi (*E. colombiana* ECLB 356, *Gl. etunicatum* LETC 236, LETC 329, LETC 455, and *Gl. mosseae* LMSS 378) are not presented as these plants remained nonmycorrhizal and behaved like noninoculated plants. Only plants colonized by *A. scrobiculata* ASCB 456 and *S. heterogama* CHTG 139 had higher shoot P concentration than *Rhizobium* control (Table 4-3). Compared with noninoculated control, single inoculation with *Gl. manihot* LMNH 980 increased shoot P concentration by 39% while *Rhizobium* had no effect. The root P concentrations of plants colonized by VA mycorrhizal fungi were all higher than their nonmycorrhizal counterparts. A maximum increase of 50% was obtained from colonization by *Gl. manihot* LMNH 980. The other isolates contributed at least a 15% increase in root P concentration. *Rhizobium* did not improve the overall P status of either mycorrhizal or nonmycorrhizal plants.

In the presence of *Rhizobium*, *Gl. manihot* LMNH 980 increased shoot P content by 712% and root P content by 1333% (Table 4-4). Mycorrhizal plants had higher P content than the nonmycorrhizal ones except those colonized by *S. pellucida* CPLC 288 which increased P content in roots but not in shoots. Effectiveness in improving P nutrition of the host varied with fungal species and isolates. *Glomus manihot* LMNH 980 was the most effective, followed by *Gi. margarita* GMRG 444, *A.*

Table 4-3. Shoot and root P concentration of *Pueraria phaseoloides* inoculated with selected VA mycorrhizal fungi and *Rhizobium* (Trial 1).

Treatment	Shoot P conc %	Root P conc %
VAMF + *Rhizobium*:		
Gl. manihot LMNH 980	0.052*bc**	0.072 b
Gi. margarita GMRG 444	0.046 d	0.055 ef
Gi. gigantea GGGT 109	0.038 e	0.067 bc
A. scrobiculata ASBC 456	0.056 ab	0.056 de
Gi. margarita GMRG 185	0.045 d	0.062 c
S. heterogama CHTG 139	0.056 b	0.055 ef
S. calospora CCLS 348	0.048 cd	0.062 c
S. pellucida CPLC 288	0.037 e	0.055 ef
VAMF or *Rhizobium*:		
Gl. manihot LMNH 980	0.061 a	0.180 a
Rhizobium	0.049 cd	0.048 g
Noninoculated	0.044 d	0.050 fg

* Means represent 9 to 12 replicates.

** Means followed by the same letter are not significantly different at $p \leq 0.05$ by Waller-Duncan K-ratio T test.

Table 4-4. Shoot and root total P content of *Pueraria phaseoloides* inoculated with selected VA mycorrhizal fungi and *Rhizobium* (Trial 1).

Treatment	Shoot P content mg		Root P content mg	
VAMF + *Rhizobium*:				
Gl. manihot LMNH 980	0.65*	a**	0.86	a
Gi. margarita GMRG 444	0.36	bc	0.34	cd
Gi. gigantea GGGT 109	0.29	d	0.40	bc
A. scrobiculata ASBC 456	0.40	b	0.29	d
Gi. margarita GMRG 185	0.32	cd	0.41	b
S. heterogama CHTG 139	0.37	b	0.30	d
S. calospora CCLS 348	0.20	e	0.18	e
S. pellucida CPLC 288	0.13	f	0.14	e
VAMF or *Rhizobium*:				
Gl. manihot LMNH 980	0.19	e	0.44	b
Rhizobium	0.08	fg	0.06	f
Noninoculated	0.06	g	0.04	f

* Means represent 9 to 12 replicates.

** Means followed by the same letter are not significantly different at $p \leq 0.05$ by Waller-Duncan K-ratio T test.

scrobiculata ASCB 456, and *S. heterogama* CHTG 139. The least effective were *S. calospora* CCLS 348 and *S. pellucida* CPLC 288. In the absence of *Rhizobium*, *Gl. manihot* LMNH 980 increased the shoot and root P content by 217% and 1000%, respectively. *Rhizobium* did not contribute to P content of nonmycorrhizal plants but stimulated shoot P content of mycorrhizal ones by 242% and root P content by 95%.

Nodulation. All isolates of mycorrhizal fungi which colonized the legume improved its nodulation (Table 4-5). However, there were differences in number and total weight of nodules due to species and isolates of the fungi. Plants colonized by *Gi. margarita* GMRG 185 and *Gi. gigantea* GGGT 109 had the most nodules. In terms of total nodule weight, *Gl. manihot* LMNH 980 was the most effective and enhanced nodulation by 1241%, followed by *Gi. gigantea* GGGT 109 and *Gi. margarita* GMRG 444. The least effective were *S. calospora* CCLS 348 and *S. pellucida* CPLC 288. *Rhizobium* inoculation failed to improve nodulation of nonmycorrhizal plants but increased the number of nodules of mycorrhizal ones by 350% and total nodule weight by 819%.

Nitrogen nutrition. The shoot N concentration of *Rhizobium*-inoculated plants was not improved by VA mycorrhizal inoculation except *S. heterogama* CHTG 139 which caused an increase of 8% (Table 4-6). However, *Gl. manihot* LMNH 980 increased shoot N concentration of plants uninoculated with *Rhizobium* by 18%. *Rhizobium*, in the absence of mycorrhiza,

Table 4-5. Nodule number and nodule weight of *Pueraria phaseoloides* inoculated with selected VA mycorrhizal fungi and *Rhizobium* (Trial 1).

Treatment	Nodule Number	Nodule Weight mg
VAMF + *Rhizobium*:		
Gl. manihot LMNH 980	27*c**	496.3 a
Gi. margarita GMRG 444	31 bc	317.4 b
Gi. gigantea GGGT 109	33 ab	306.0 b
A. scrobiculata ASCB 456	21 d	249.8 c
Gi. margarita GMRG 185	37 a	233.7 c
S. heterogama CHTG 139	21 d	249.2 c
S. calospora CCLS 348	17 d	126.4 d
S. pellucida CPLC 288	16 d	109.7 d
VAMF or *Rhizobium*:		
Gl. manihot LMNH 980	6 e	53.9 e
Rhizobium	8 e	36.7 e
Noninoculated	4 e	14.2 e

* Means represent 9 to 12 replicates.

** Means with the same letter are not significantly different at $p \leq 0.05$ by Waller-Duncan K-ratio T test.

Table 4-6. Shoot and root N concentration of *Pueraria phaseoloides* inoculated with selected VA mycorrhizal fungi and *Rhizobium* (Trial 1).

Treatment	Shoot N conc %	Root N conc %
VAMF + *Rhizobium*:		
Gl. manihot LMNH 980	1.47*bc**	1.21 ab
Gi. margarita GMRG 444	1.37 d	1.11 d
Gi. gigantea GGGT 109	1.27 e	1.14 cd
A. scrobiculata ASCB 456	1.49 b	1.17 bc
Gi. margarita GMRG 185	1.36 de	1.11 d
S. heterogama CHTG 139	1.61 a	1.16 cd
S. calospora CCLS 348	1.38 cd	1.14 cd
S. pellucida CPLC 288	1.32 de	1.22 ab
VAMF or *Rhizobium*:		
Gl. manihot LMNH 980	1.51 b	1.26 a
Rhizobium	1.49 b	1.25 a
Noninoculated	1.28 e	1.19 bc

* Means represent 9 to 12 replicates.

** Means followed by the same letter are not significantly different at $p \leq 0.05$ by Waller-Duncan K-ratio T test.

elevated shoot N concentration by 16%. The shoot N concentration of plants with both *Gl. manihot* LMNH 980 and *Rhizobium* was not better than those colonized singly by either symbiont. Colonization by VA mycorrhizal fungi did not improve the root N concentration of rhizobial plants nor did *Rhizobium* improve the root N concentration of mycorrhizal plants. Single symbiosis involving *Gl. manihot* LMNH 980 or *Rhizobium* increased root N concentration by at least 5% relative to noninoculated control.

Except for those isolates which failed to colonize *P. phaseoloides* in the test soil, all the other isolates evaluated improved the N content of the host (Table 4-7). *Glomus manihot* LMNH 980 caused the greatest increase in shoot and root N content equivalent to 636% and 1164%, respectively. Its effect was different from that of any other isolate. *Gigaspora margarita* GMRG 444, GMRG 185, *Gi. gigantea* GGGT 109, *S. heterogama* CHTG 139, and *A. scrobiculata* ASCB 456 also improved shoot and root N content. These treatments did not differ from each other in regard to their effects on shoot total N except GMRG 185, and in regard to root total N except CHTG 139. The least effective VA mycorrhizal fungi were *S. calospora* CCLS 348 and *S. pellucida* CPLC 288. Nevertheless, N content of plants with either of these isolates was better than that of the corresponding control. *Rhizobium* did not improve the N content of nonmycorrhizal *P. phaseoloides*. However, *Rhizobium* increased the shoot and root total N of

Table 4-7. Shoot and root total N content of *Pueraria phaseoloides* inoculated with selected VA mycorrhizal fungi and *Rhizobium* (Trial 1).

Treatment	Shoot N content mg	Root N content mg
VAMF + *Rhizobium*:		
Gl. manihot LMNH 980	18.39*a**	14.84 a
Gi. margarita GMRG 444	10.61 b	6.80 bc
Gi. gigantea GGGT 109	9.64 bc	6.77 bc
A. scrobiculata ASBC 456	10.44 b	6.06 c
Gi. margarita GMRG 185	9.09 c	7.42 b
S. heterogama CHTG 139	10.67 b	6.43 bc
S. calospora CCLS 348	5.90 d	3.47 d
S. pellucida CPLC 288	4.71 d	3.00 d
VAMF or *Rhizobium*:		
Gl. manihot LMNH 980	4.76 d	3.13 d
Rhizobium "EL"	2.46 e	1.44 e
Noninoculated	1.63 e	0.92 f

* Means represent 9 to 12 replicates.

** Means followed by the same letter are not significantly different at $p \leq 0.05$ by Waller-Duncan K-ratio T test.

mycorrhizal plants by as much as 283% and 381%, respectively. Root colonization by *Gl. manihot* LMNH 980 alone increased shoot and root N content.

Growth response. Among those also inoculated with *Rhizobium*, the effects of *Gl. manihot* LMNH 980, *Gi. margarita* GMRG 444, *Gi. gigantea* GGGT 109, *A. scrobiculata* ASCB 456, *Gi. margarita* GMRG 185, *S. heterogama* CHTG 139, *S. calospora* CCLS 348, and *S. pellucida* CPLC 288 on shoot and root dry weights were all significant (Table 4-8). The best growth response was obtained from *Gl. manihot* LMNH 980 which gave as much as 293% and 414% increases in shoot and root dry weights, respectively. The least response was obtained from *S. calospora* CCLS 348 and *S. pellucida* CPLC 288. Comparing the two isolates of *Gi. margarita*, GMRG 444 was better than GMRG 185. There was no response to inoculation with *Gl. etunicatum* LETC 236, LETC 329, and LETC 455, *Gl. mosseae* LMSS 378, and *E. colombiana* ECLB 356, since these fungi failed to colonize *P. phaseoloides* in Pacolet soil presumably due to Al. In the absence of *Rhizobium*, *Gl. manihot* LMNH 980 increased the shoot and root dry weights of the legume by 149% and 212%, respectively. However, *Rhizobium* alone did not improve the plant dry weight.

Plant height was also affected by VA mycorrhizal colonization (Table 4-9). Comparing the different isolates of VA mycorrhizal fungi, *Gl. manihot* LMNH 980, *Gi. margarita* GMRG 444, *Gi. gigantea* GGGT 109, *A. scrobiculata* ASCB 456, and *Gi.*

Table 4-8. Shoot and root dry weights of *Pueraria phaseoloides* inoculated with selected VA mycorrhizal fungi and *Rhizobium* (Trial 1).

Treatment	Shoot Dry Weight mg		Root Dry Weight mg	
VAMF + *Rhizobium*:				
Gl. manihot LMNH 980	1390*	a**	1363	a
Gi. margarita GMRG 444	866	b	673	bc
Gi. gigantea GGGT 109	836	bc	654	bc
A. scrobiculata ASBC 456	779	bcd	569	c
Gi. margarita GMRG 185	764	cd	730	b
S. heterogama CHTG 139	734	d	611	bc
S. calospora CCLS 348	478	e	333	d
S. pellucida CPLC 288	398	ef	268	de
VAMF + *Rhizobium*:				
Gl. manihot LMNH 980	354	f	265	de
Rhizobium	185	g	128	f
Noninoculated	142	g	85	f

* Means represent 9 to 12 replicates.

** Means followed by the same letter are not significantly different at $p \leq 0.05$ by Waller-Duncan K-ratio T test.

Table 4-9. Height and root collar diameter of *Pueraria phaseoloides* inoculated with selected VA mycorrhizal fungi and *Rhizobium* (Trial 1).

Treatment	Plant Height cm	Root Collar Diameter mm
VAMF + *Rhizobium*:		
Gl. manihot LMNH 980	47.6*a**	3.0 b
Gi. margarita GMRG 444	38.1 b	2.8 bc
Gi. gigantea GGGT 109	13.1 cd	3.4 a
A. scrobiculata ASBC 456	19.3 c	2.6 cd
Gi. margarita GMRG 185	19.0 c	3.0 b
S. heterogama CHTG 139	9.6 de	2.9 b
S. calospora CCLS 348	6.0 de	2.4 de
S. pellucida CPLC 288	5.5 de	2.4 de
VAMF + *Rhizobium*:		
Gl. manihot LMNH 980	5.6 de	2.2 ef
Rhizobium	4.3 e	1.9 g
Noninoculated	3.6 e	1.4 h

* Means represent 9 to 12 replicates.

** Means followed by the same letter are not significantly different at $p \leq 0.05$ by Waller-Duncan K-ratio T test.

margarita GMRG 185 had effects on height of *P. phaseoloides* but not *S. heterogama* CHTG 139, *S. calospora* CCLS 348 and *S. pellucida* CPLC 288. Plants colonized by *Gl. manihot* LMNH 980 were the tallest, followed by those with *Gi. margarita* GMRG 444. The effects of *Gi. gigantea* GGGT 109, *A. scrobiculata* ASCB 456 and *Gi. margarita* GMRG 185 were not different from each other. Between isolates of *Gi. margarita*, GMRG 444 promoted host growth better than GMRG 185. *Pueraria phaseoloides* did not benefit from single symbiosis with either *Gl. manihot* LMNH 980 or *Rhizobium*, with respect to plant height growth.

Plant diameter was increased by VA mycorrhizal fungi (Table 4-9). Those colonized by *Gi. gigantea* GGGT 109 had the largest diameter, followed by those with *Gl. manihot* LMNH 980, *Gi. margarita* GMRG 444 and GMRG 185, and *S. heterogama* CHTG 139. Although plants colonized by *A. scrobiculata* ASCB 456, *S. calospora* CCLS 348, and *S. pellucida* CPLC 288 had the smallest diameter among the mycorrhizal ones, the values were higher than the *Rhizobium* control. Either *Gl. manihot* LMNH 980 or *Rhizobium* improved the diameter of plants by 57% and 36%, respectively over the noninoculated ones.

Correlations. Nodulation and mycorrhizal colonization were significantly correlated with growth variables, with nodule weight being more correlated than nodule number (Table 4-10). Moreover, nodulation and VA mycorrhizal colonization had high significant correlations with N and P content, but

Table 4-10. Pearson coefficients for correlating nodulation and root VAM colonization with various growth and nutritional variables in *Pueraria phaseoloides* (Trial 1).

Variable	Variable		
	Nodule Number	Nodule Weight	Root VAM Colonization***
Shoot Fresh Weight	0.74*	0.92	0.89
Shoot Dry Weight	0.73	0.92	0.88
Root Fresh Weight	0.73	0.88	0.82
Root Dry Weight	0.67	0.84	0.78
Shoot N Concentration	-0.21	ns	ns
Shoot Total N Content	0.68	0.90	0.85
Root N Concentration	-0.35	-0.33	-0.35
Root Total N Content	0.66	0.85	0.78
Shoot P Concentration	-0.27	ns	ns
Shoot Total P Content	0.63	0.87	0.82
Root P Concentration	ns**	ns	0.30
Root Total P Content	0.59	0.78	0.79

* Coefficients are obtained from correlation analysis involving 183 observations per variable.

** ns indicates that correlation is not significant at $p \leq 0.01$.

*** Pearson coefficients for correlating nodule number and nodule weight with root VAM colonization are 0.73 and 0.84, respectively.

had either negative or insignificant correlations with N and P concentration. The only exception was that root P concentration was correlated with root colonization. Shoot and root dry weights were highly correlated with N and P content, negatively correlated with root N concentration, and not correlated at all with either shoot N concentration or P concentration (Table 4-11). Phosphorus content was highly correlated with N content (Table 4-12). Furthermore, shoot P concentration was correlated with shoot N concentration, and root P concentration was correlated with root N concentration.

Pueraria phaseoloides (Trial 2)

Root VA mycorrhizal colonization. Additional isolates of VA mycorrhizal fungi were evaluated in trial 2. These included *Gi. gigantea* GGGT 663 and *S. calospora* CCLS 269. Two isolates of *Gl. etunicatum* LETC 329 and LETC 455 which were evaluated in trial 1 were not included in trial 2. The results obtained from trial 1 agree with that from trial 2 (Table 4-13). Only two isolates gave inconsistent results. *Acaulospora scrobiculata* ASCB 456 colonized *P. phaseoloides* in trial 1 but not in trial 2. On the other hand, *E. colombiana* ECLB 356 failed to colonize the legume in trial 1 but did colonize the host in trial 2. Root colonization by *Gl. manihot* LMNH 980 was greater than that caused by any other isolate. Comparing isolates of the same species, *Gi. gigantea* GGGT 109 colonized the host more extensively than did GGGT

Table 4-11. Pearson coefficients for correlating shoot and root dry weights with N and P nutrition of *Pueraria phaseoloides* (Trial 1).

Variable	Variable	
	Shoot Dry Weight mg	Root Dry Weight mg
Shoot N concentration	ns**	ns
Shoot total N content	0.98	0.93
Root N concentration	-0.36*	-0.36
Root total N content	0.93	0.99
Shoot P concentration	ns	ns
Shoot total P content	0.96	0.90
Root P concentration	ns	ns
Root total P content	0.86	0.91

* Coefficients are obtained from correlation analysis involving 183 observations per variable.

** ns indicates that correlation is not significant at $p \leq 0.01$.

Table 4-12. Pearson coefficients for correlating P nutrition with N nutrition of *Pueraria phaseoloides* (Trial 1).

Variable	Variable			
	Shoot P conc	Shoot P content	Root P conc	Root P content
Shoot N conc	0.49*	ns	ns	ns
Shoot N content	ns**	0.97	ns	0.86
Root N conc	ns	-0.34	0.20	ns
Root N content	ns	0.94	ns	0.94

* Coefficients are obtained from correlation analysis involving 183 observations per variable.

** ns indicates that correlation is not significant at $p \leq 0.01$.

Table 4-13. Root VAM colonization of *Pueraria phaseoloides* inoculated with selected VA mycorrhizal fungi and *Rhizobium* (Trial 2).

Treatment	Root VAM Colonization %
VAMF + *Rhizobium*:	
Gl. manihot LMNH 980	77.0*a**
Gi. gigantea GGGT 109	57.1 bc
Gi. margarita GMRG 185	54.0 c
E. colombiana ECLB 356	40.4 d
Gi. margarita GMRG 444	60.9 b
Gi. gigantea GGGT 663	42.0 d
S. heterogama CHTG 139	40.6 d
S. calospora CCLS 348	30.4 e
S. pellucida CPLC 288	32.7 e
S. calospora CCLS 269	0.0 f
A. scrobiculata ASBC 456	0.0 f
Gl. etunicatum LETC 236	0.0 f
Gl. mosseae LMSS 378	0.0 f
Rhizobium	0.0 f
Noninoculated	0.0 f

* Means represent 4 to 8 replicates.

** Means with the same letter are not significantly different at $p \leq 0.05$ by Waller-Duncan K-ratio T test.

663; *S. calospora* CCLS 348 colonized the legume but CCLS 269 did not. Again, *Gi. margarita* GMRG 444 performed better than GMRG 185.

Phosphorus nutrition. The P concentration of *P. phaseoloides* was affected by VA mycorrhizal fungi (Table 4-14). *Scutellispora pellucida* CPLC 288 caused the greatest increase in shoot P concentration and its effect was different from all the other isolates. *Gigaspora gigantea* GGGT 109, *S. calospora* CCLS 348, and *Gi. margarita* GMRG 185 also improved the shoot P concentration. These treatments, however, did not differ from each other. Minimum response was obtained from *E. colombiana* ECLB 356 which had nevertheless improved shoot P concentration by 9%. As in trial 1, the shoot P concentration of plants colonized by *Gi. margarita* GMRG 444 and *Gl. manihot* LMNH 980 was not different or even lower than that of the *Rhizobium* control.

Root P concentration was increased by root VA mycorrhizal colonization in both trials. *Glomus manihot* LMNH 980 was better than any other treatment. This was followed by *Gi. gigantea* GGGT 663 and GGGT 109, and *Gi. margarita* GMRG 444 which were more effective than *E. colombiana* ECLB 356, *S. calospora* CCLS 348, and *S. pellucida* CPLC 288.

Mycorrhizal fungi improved the total amount of P taken up by *P. phaseoloides* (Table 4-15). The most effective fungi in this regard were *Gl. manihot* LMNH 980, *Gi. gigantea* GGGT 109, and *Gi. margarita* GMRG 185 which increased shoot total P

Table 4-14. Shoot and root P concentration of *Pueraria phaseoloides* inoculated with selected VA mycorrhizal fungi and *Rhizobium* (Trial 2).

Treatment	Shoot P conc %	Root P conc %
VAMF + *Rhizobium*:		
Gl. manihot LMNH 980	0.064*g**	0.090 a
Gi. gigantea GGGT 109	0.085 b	0.070 c
Gi. gigantea GMRG 185	0.083 bc	0.061 de
E. colombiana ECLB 356	0.075 d	0.056 ef
Gi. margarita GMRG 444	0.065 fg	0.066 cd
Gi. gigantea GGGT 663	0.080 c	0.076 b
S. heterogama CHTG 139	0.071 de	0.063 d
S. calospora CCLS 348	0.085 b	0.054 f
S. pellucida CPLC 288	0.098 a	0.046 g
Rhizobium	0.069 ef	0.033 h
Noninoculated	0.083 bc	0.037 h

* Means represent 4 to 8 replicates.

** Means followed by the same letter are not significantly different at $p \leq 0.05$ by Waller-Duncan K-ratio T test.

Table 4-15. Shoot and root total P content of *Pueraria phaseoloides* inoculated with selected VA mycorrhizal fungi and *Rhizobium* (Trial 2).

Treatment	Shoot P Content mg	Root P Content mg
VAMF + *Rhizobium*:		
Gl. manihot LMNH 980	0.405*a**	0.438 a
Gi. gigantea GGGT 109	0.426 a	0.261 b
Gi. gigantea GMRG 185	0.380 ab	0.196 cd
E. colombiana ECLB 356	0.342 bc	0.174 cd
Gi. margarita GMRG 444	0.277 de	0.203 cd
Gi. gigantea GGGT 663	0.300 cd	0.222 bc
S. heterogama CHTG 139	0.243 e	0.160 d
S. calospora CCLS 348	0.168 f	0.077 e
S. pellucida CPLC 288	0.168 f	0.038 e
Rhizobium	0.067 g	0.033 e
Noninoculated	0.061 g	0.059 e

* Means represent 4 to 8 replicates.

** Means followed by the same letter are not significantly different at $p \leq 0.05$ by Waller-Duncan K-ratio T test.

content by at least 467%. This was followed by *E. colombiana* ECLB 356 and then by *Gi. gigantea* GGGT 663 and *Gi. margarita* GMRG 444. The least response was obtained from *S. calospora* CCLS 348 and *S. pellucida* CPLC 288 which gave a 151% increase in shoot P content. In this trial, plants colonized by *Gi. margarita* GMRG 185 had higher shoot total P than those with GMRG 444.

Root total P content was also affected by VA mycorrhizal fungi where *Gl. manihot* LMNH 980 increased the amount by 1227%. Plants colonized by *Gigaspora* isolates had higher root P content than those colonized by *Scutellispora*. *Gigaspora margarita* GMRG 185 and GMRG 444 performed equally well. *Entrophospora colombiana* ECLB 356 increased root P by 427% while *S. calospora* CCLS 348 and *S. pellucida* CPLC 288 did not have any effect.

Nodulation. In contrast to the results of trial 1 where all isolates of VA mycorrhizal fungi which colonized the legume improved its nodulation, *S. calospora* CCLS 348 and *S. pellucida* CPLC 288 did not affect nodulation in trial 2 (Table 4-16). The other VA mycorrhizal fungi enhanced nodulation. Plants colonized by *Gi. margarita* GMRG 185 and *E. colombiana* ECLB 356 were the most nodulated, with at least 417% more nodules than the *Rhizobium* control plants. Less nodules were developed in plants inoculated with *Gi. gigantea* GGGT 109 and *S. heterogama* CHTG 139. Those colonized by *Gl.*

Table 4-16. Nodule number and nodule weight of *Pueraria phaseoloides* inoculated with selected VA mycorrhizal fungi and *Rhizobium* (Trial 2).

Treatment	Nodule Number	Nodule Weight mg
VAMF + *Rhizobium*:		
Gl. manihot LMNH 980	28*bc**	66.5 a
Gi. gigantea GGGT 109	22 c	56.8 ab
Gi. margarita GMRG 185	37 a	66.8 a
E. colombiana ECLB 356	31 ab	52.5 bc
Gi. margarita GMRG 444	23 bc	35.0 de
Gi. gigantea GGGT 663	24 bc	40.6 cd
S. heterogama CHTG 139	21 cd	24.1 ef
S. calospora CCLS 348	14 de	16.4 fg
S. pellucida CPLC 288	9 ef	10.5 g
Rhizobium	6 ef	3.8 g
Noninoculated	3 f	5.2 g

* Means represent 4 to 8 replicates.

** Means with the same letter are not significantly different at $p<0.05$ by Waller-Duncan K-ratio T test.

manihot LMNH 980, *Gi. margarita* GMRG 444, and *Gi. gigantea* GGGT 663 had intermediate number of nodules.

In terms of total nodule weight per plant, those with *Gl. manihot* LMNH 980, *Gi. gigantea* GGGT 109, and *Gi. margarita* GMRG 185 were better than those with *Gi. margarita* GMRG 444, *Gi. gigantea* GGGT 663, and *S. heterogama* CHTG 139. Again, *Glomus manihot* LMNH 980 proved the most effective isolate in increasing nodule weight, 943% greater than the *Rhizobium* control. *Rhizobium*, in the absence of VA mycorrhizal fungi, did not affect nodulation, whether in terms of nodule number or nodule weight.

Nitrogen nutrition. The Al-tolerant mycorrhizal fungi increased the N concentration of the legume (Table 4-17). Plants colonized by *S. calospora* CCLS 348 and *S. pellucida* CPLC 288 had higher shoot N concentration than those colonized by *E. colombiana* ECLB 356, *Gi. margarita* GMRG 185, GMRG 444, *Gl. manihot* LMNH 980, and *S. heterogama* CHTG 139. Plants with *Gi. gigantea* GGGT 109 and GGGT 663 had lower shoot N concentration than those inoculated with the other mycorrhizal fungi, but higher than that of the *Rhizobium* control.

In regards to effect on root N concentration, *Gi. margarita* GMRG 185, *Gi. gigantea* GGGT 663, *Gl. manihot* LMNH 980, *Gi. gigantea* GGGT 109, and *S. pellucida* CPLC 288 were better than *Gi. margarita* GMRG 444, *E. colombiana* ECLB 356, and *S. calospora* CCLS 348. Plants colonized by *S. heterogama* CHTG 139 had the least root N concentration.

Table 4-17. Shoot and root N concentration of *Pueraria phaseoloides* inoculated with selected VA mycorrhizal fungi and *Rhizobium* (Trial 2).

Treatment	Shoot N conc %	Root N conc %
VAMF + *Rhizobium*:		
Gl. manihot LMNH 980	2.19*c**	1.48 bc
Gi. gigantea GGGT 109	2.05 e	1.46 bc
Gi. margarita GMRG 185	2.19 c	1.54 a
E. colombiana ECLB 356	2.22 b	1.38 ef
Gi. margarita GMRG 444	2.19 c	1.39 de
Gi. gigantea GGGT 663	2.13 d	1.48 b
S. heterogama CHTG 139	2.18 c	1.18 h
S. calospora CCLS 348	2.30 a	1.37 ef
S. pellucida CPLC 288	2.27 a	1.44 c
Rhizobium	1.97 f	1.23 g
Noninoculated	2.12 d	1.40 de

* Means represent 4 to 8 replicates.

** Means with the same letter are not significantly different at $p \leq 0.05$ by Waller-Duncan K-ratio T test.

Total N in shoots and roots of *P. phaseoloides* were affected by VA mycorrhizal fungi (Table 4-18). As with P content, shoot and root N content were highest in plants colonized by *Gl. manihot* LMNH 980. As much as 637% and 500% increases were obtained, respectively. This was followed by *Gigaspora* isolates *Gi. gigantea* GGGT 109 and GGGT 663, *Gi. margarita* GMRG 185 and GMRG 444, and then by *E. colombiana* ECLB 356. There was no difference between *Gi. margarita* GMRG 185 and GMRG 444 but there was a difference between *Gi. gigantea* GGGT 109 and GGGT 663 in terms of shoot N content. In general, *Scutellispora* species were found less effective than *Gigaspora* species in this study. Within *Scutellispora*, *S. heterogama* CHTG 139 was better than *S. pellucida* CPLC 288. As to the effect of *Rhizobium*, the plants did not benefit from the bacteria in the absence of mycorrhizal fungi.

Growth response. Selected VA mycorrhizal fungi again affected the growth performance of *P. phaseoloides* in trial 2 (Table 4-19). The most effective isolate *Gl. manihot* LMNH 980 increased shoot dry weight by 599% and root dry weight by 424%. *Gigaspora gigantea* GGGT 109, *Gi. margarita* GMRG 185, and *E. colombiana* ECLB 356 were less effective than *Gl. manihot* LMNH 980 but more effective than *S. calospora* CCLS 348 and *S. pellucida* CPLC 288. The latter two isolates increased shoot dry weight by at least 74% but did not affect root dry weight. With respect to the performance of different isolates of the same species, the effects of *Gi. margarita* GMRG 185 and

Table 4-18. Shoot and root total N content of *Pueraria phaseoloides* inoculated with selected VA mycorrhizal fungi and *Rhizobium* (Trial 2).

Treatment	Shoot N Content mg	Root N Content mg
VAMF + *Rhizobium*:		
Gl. manihot LMNH 980	14.0*a**	7.2 a
Gi. gigantea GGGT 109	10.3 b	5.3 b
Gi. gigantea GMRG 185	10.0 b	5.0 b
E. colombiana ECLB 356	10.2 b	4.3 bc
Gi. margarita GMRG 444	9.3 bc	4.3 bc
Gi. gigantea GGGT 663	8.1 cd	4.3 bc
S. heterogama CHTG 139	7.5 d	3.0 cd
S. calospora CCLS 348	4.5 e	1.0 de
S. pellucida CPLC 288	3.9 e	1.2 e
Rhizobium	1.9 f	1.2 e
Noninoculated	1.6 f	2.2 de

* Means represent 4 to 8 replicates.

** Means followed by the same letter are not significantly different at $p \leq 0.05$ by Waller-Duncan K-ratio T test.

Table 4-19. Shoot and root dry weights of *Pueraria phaseoloides* inoculated with selected VA mycorrhizal fungi and *Rhizobium* (Trial 2).

Treatment	Shoot Dry Weight mg	Root Dry Weight mg
VAMF + *Rhizobium*:		
Gl. manihot LMNH 980	707.2*a**	534.5 a
Gi. gigantea GGGT 109	556.6 b	402.1 b
Gi. gigantea GMRG 185	509.8 bc	355.5 bc
E. colombiana ECLB 356	508.1 bc	345.6 bc
Gi. margarita GMRG 444	472.0 cd	336.0 bc
Gi. gigantea GGGT 663	422.9 de	320.4 bc
S. heterogama CHTG 139	379.6 e	277.2 cd
S. calospora CCLS 348	219.3 f	155.0 de
S. pellucida CPLC 288	188.3 f	90.7 e
Rhizobium	108.1 g	110.5 e
Noninoculated	82.1 g	175.2 de

* Means represent 4 to 8 replicates.

** Means followed by the same letter are not significantly different at $p \leq 0.05$ by Waller-Duncan K-ratio T test.

GMRG 444 did not differ from each other both in terms of shoot dry weight and root dry weight. *Rhizobium* did not affect the growth of nonmycorrhizal plants as observed in trial 1.

Correlations. Nodule number, nodule weight, and root VA mycorrhizal colonization were correlated with growth and nutritional variables except with shoot P concentration (Table 4-20). Mycorrhizal colonization was more highly correlated with growth parameters than were nodule number and nodule weight. Shoot and root N concentration were less correlated to nodulation and root mycorrhizal colonization than were shoot and root total N content. The same relationship was found true for P concentration and P content.

Shoot and root dry weights were highly correlated with N and P content (Table 4-21). Correlation between the growth variables and N or P concentration was low except for root P concentration. There were low correlations between N and P concentrations (Table 4-22). However, shoot P content and root P content were highly correlated with either shoot N content or root N content.

Stylosanthes guianensis (Trial 1)

Root VA mycorrhizal colonization. Percent colonization of *S. guianensis* roots by VA mycorrhizal fungi differed with species and isolates of the fungal symbiont (Table 4-23). *Glomus manihot* LMNH 980 colonized the host most extensively, followed by *Gigaspora* species *Gi. gigantea* GGGT 109, *Gi.*

Table 4-20. Pearson coefficients for correlating nodulation and root VAM colonization with various growth and nutritional variables in *Pueraria phaseoloides* (Trial 2).

Variable	Variable		
	Nodule Number	Nodule Weight	Root VAM*** Colonization
Shoot fresh weight	0.82*	0.84	0.90
Shoot dry weight	0.82	0.85	0.89
Root fresh weight	0.70	0.76	0.82
Root dry weight	0.63	0.68	0.69
Shoot N conc	0.36	0.30	0.49
Shoot N content	0.83	0.85	0.90
Root N conc	0.33	0.47	0.39
Root N content	0.64	0.70	0.70
Shoot P conc	ns**	ns	ns
Shoot P content	0.81	0.86	0.88
Root P conc	0.53	0.61	0.70
Root P content	0.65	0.74	0.79

* Coefficients are obtained from correlation analysis involving 105 observations per variable.

** ns indicates that correlation is not significant at $p \leq 0.01$.

*** Pearson coefficient for correlating nodule number root VAM colonization 0.73.

Table 4-21. Pearson coefficients for correlating shoot and root dry weights with N and P nutrition of *Pueraria phaseoloides* (Trial 2).

Variable	Variable	
	Shoot Dry Weight mg	Root Dry Weight mg
Shoot N concentration	0.33*	ns
Shoot total N content	0.99	0.79
Root N concentration	0.37	0.29
Root total N content	0.80	0.99
Shoot P concentration	ns**	ns
Shoot total P content	0.98	0.77
Root P concentration	0.69	0.54
Root total P content	0.87	0.93

* Coefficients are obtained from correlation analysis involving 105 observations per variable.

** ns indicates that correlation is not significant at $p \leq 0.01$.

Table 4-22. Pearson coefficients for correlating P nutrition with N nutrition of *Pueraria phaseoloides* (Trial 2).

Variable	Variable			
	Shoot P Conc	Shoot P Content	Root P Conc	Root P Content
Shoot N Conc	0.38*	0.33	0.26	ns
Shoot N Content	ns**	0.97	0.69	0.87
Root N Conc	0.40	0.43	0.38	0.37
Root N Content	ns	0.78	0.56	0.94

* Coefficients are obtained from correlation analysis involving 105 observations per variable.

** ns indicates that correlation is not significant at $p \leq 0.01$.

Table 4-23. Root mycorrhizal colonization in *Stylosanthes guianensis* inoculated with selected VA mycorrhizal fungi and *Rhizobium* (Trial 1).

Treatment	Root Mycorrhizal Colonization %
VAMF + *Rhizobium*:	
Gl. manihot LMNH 980	81.5*a**
Gi. gigantea GGGT 109	62.7 b
Gi. margarita GMRG 185	57.8 c
Gi. margarita GMRG 444	62.3 b
S. heterogama CHTG 139	41.9 e
S. calospora CCLS 348	47.2 d
S. pellucida CPLC 288	44.4 de
A. scrobiculata ASBC 456	44.0 de
E. colombiana ECLB 356	0.0 f
Gl. etunicatum LETC 236	0.0 f
Gl. etunicatum LETC 329	0.0 f
Gl. mosseae LMSS 378	0.0 f
VAMF or *Rhizobium*:	
Gl. manihot LMNH 980	57.0 c
Rhizobium	0.0 f
Noninoculated	0.0 f

* Means followed by the same letter are not significantly different at $p \leq 0.05$ by Waller-Duncan K-ratio T test.

** Means represent 12 to 18 replicates.

margarita GMRG 444, and *Gi. margarita* GMRG 185. Colonization by *Gi. margarita* GMRG 444 was better than by GMRG 185. Plants inoculated with *Scutellispora* species were less colonized than those with *Gigaspora* species. Within *Scutellispora*, colonization by *S. calospora* CCLS 348 was the highest, better than *S. heterogama* CHTG 139 but not different from *S. pellucida* CPLC 288. Root colonization by *Gl. manihot* LMNH 980 in the presence of *Rhizobium* was greater by 43% than in the absence of *Rhizobium*. *Entrophospora colombiana* ECLB 356, *Gl. mosseae* LMSS 378, *Gl. etunicatum* LETC 236, and LETC 329 were unable to colonize *S. guianensis* in Pacolet soil. *Rhizobium* and noninoculated control seedlings remained nonmycorrhizal.

Phosphorus nutrition. Colonization by VA mycorrhizal fungi increased the shoot P concentration of *S. guianensis* (Table 4-24). The indigenous isolate *Gl. manihot* LMNH 980 which colonized the host most extensively, increased shoot P concentration most by 187%. There was no discernible superiority of *Gigaspora* over *Scutellispora*. Plants colonized by *Gi. gigantea* GGGT 109 had similar shoot P concentration with those colonized by *S. heterogama* CHTG 139 and *S. calospora* CCLS 348. The least shoot P concentration was found in plants with *S. pellucida* CPLC 288; 61% greater than *Rhizobium* control. Plants inoculated with *Rhizobium* had lower shoot P concentration than their uninoculated counterparts, whether mycorrhizal or not. On the other hand, *Gl. manihot*

Table 4-24. Shoot and root P concentration of *Stylosanthes guianensis* inoculated with selected VA mycorrhizal fungi and *Rhizobium* (Trial 1).

Treatment	Shoot P conc %	Root P conc %
VAMF + *Rhizobium*:		
Gl. manihot LMNH 980	0.066*b**	0.054 b
Gi. gigantea GGGT 109	0.057 cd	0.046 cd
Gi. gigantea GMRG 185	0.048 e	0.044 d
Gi. margarita GMRG 444	0.050 e	0.037 ef
S. heterogama CHTG 139	0.057 cd	0.038 ef
S. calospora CCLS 348	0.061 bc	0.045 cd
S. pellucida CPLC 288	0.037 f	0.048 cd
A. scrobiculata ASCB 456	0.051 de	0.039 e
VAMF + *Rhizobium*:		
Gl. manihot LMNH 980	0.105 a	0.074 a
Rhizobium	0.023 h	0.053 b
Noninoculated	0.029 g	0.050 bc

* Means represent 12 to 18 replicates.

** Means followed by the same letter are not significantly different at $p \leq 0.05$ by Waller-Duncan K-ratio T test.

LMNH 980 raised the P concentration of the legume regardless of *Rhizobium*.

The VA mycorrhizal fungi did not improve root P concentration. Except those colonized by *Gl. manihot* LMNH 980, other mycorrhizal plants had lower root P concentration than the *Rhizobium* control. *Rhizobium* had no significant effect on root P concentration of either mycorrhizal or nonmycorrhizal plants.

Root VA mycorrhizal colonization increased the shoot and root P content of *S. guianensis* (Table 4-25). *Glomus manihot* LMNH 980 was the most effective in enhancing P content. *Gigaspora gigantea* GGGT 109 and *Gi. margarita* GMRG 185 were the next effective ones. The P status of seedlings inoculated with *Scutellispora* species was inferior to those with *Gigaspora* species. The P content of roots colonized by *S. heterogama* CHTG 139 or *S. calospora* CCLS 348 was higher than that colonized by *S. pellucida* CPLC 288. In terms of effect on shoot P content, *A. scrobiculata* ASCB 456 was less efffective than *Scutellispora* species.

Nodulation. The VA mycorrhizal fungi evaluated improved nodulation of *S. guianensis* by *Rhizobium* (Table 4-26). Plants colonized by *Gl. manihot* LMNH 980 had the most nodules, 440% higher than that of the *Rhizobium* control. This was followed by *Gigaspora* species. *Gigaspora margarita* GMRG 185 and *Gi. gigantea* GGGT 109 were better than GMRG 444. Nodulation in plants with *Scutellispora* was less than those with *Gigaspora*

Table 4-25. Shoot and root total P content of *Stylosanthes guianensis* inoculated with selected VA mycorrhizal fungi and *Rhizobium* (Trial 1).

Treatment	Shoot P content mg	Root P content mg
VAMF + *Rhizobium*:		
Gl. manihot LMNH 980	0.607*a**	0.301 a
Gi. gigantea GGGT 109	0.434 b	0.188 c
Gi. gigantea GMRG 185	0.365 c	0.217 b
Gi. margarita GMRG 444	0.338 cd	0.132 de
S. heterogama CHTG 139	0.321 d	0.110 e
S. calospora CCLS 348	0.313 d	0.136 d
S. pellucida CPLC 288	0.169 e	0.133 de
A. scrobiculata ASCB 456	0.161 e	0.073 f
VAMF + *Rhizobium*:		
Gl. manihot LMNH 980	0.184 e	0.076 f
Rhizobium	0.012 f	0.021 g
Noninoculated	0.015 f	0.024 g

* Means represent 12 to 18 replicates.

** Means followed by the same letter are not significantly different at $p \leq 0.05$ by Waller-Duncan K-ratio T test.

Table 4-26. Number of nodules in *Stylosanthes guianensis* inoculated with selected VA mycorrhizal fungi and *Rhizobium* (Trial 1).

Treatment	Number of Nodules
VAMF + *Rhizobium*:	
Gl. manihot LMNH 980	27*a**
Gi. gigantea GGGT 109	21 b
Gi. margarita GMRG 185	23 b
Gi. margarita GMRG 444	17 c
S. heterogama CHTG 139	15 cd
S. calospora CCLS 348	23 b
S. pellucida CPLC 288	12 d
A. scrobiculata ASBC 456	14 d
VAMF or *Rhizobium*:	
Gl. manihot LMNH 980	8 g
Rhizobium	5 ef
Noninoculated	4 g

* Means represent 12 to 18 replicates.

** Means followed by the same letter are not significantly different at $p \leq 0.05$ by Waller-Duncan K-ratio T test.

species, except *S. calospora* CCLS 348. The effect of *S. heterogama* CHTG 139 was not different from *S.pellucida* CPLC 288. Plants colonized by *A. scrobiculata* ASCB 456 had the least nodules among the mycorrhizal seedlings but had more than the *Rhizobium* control. *Rhizobium* inoculation alone increased nodule number by 25%, *Gl. manihot* LMNH 980 alone by 100%, and together by 575%, relative to uninoculated control. Seedlings not inoculated with *Rhizobium* became nodulated probably by indigenous soil rhizobia.

Nitrogen nutrition. The shoot N concentration of *S. guianensis* was increased by VA mycorrhizal fungi (Table 4-27). The greatest increases equivalent to 31% and 24% were obtained from *Gl. manihot* LMNH 980 and *A. scrobiculata* ASCB 456, respectively. *Gigaspora* isolates did not perform any better than *Scutellispora*. For instance, the effect of GGGT 109 did not differ from that of CCLS 348; the effects of *Gi. margarita* GMRG 185 or GMRG 444 did not differ from those of *S. pellucida* CPLC 288 or *S. heterogama* CHTG 139. However, there were differences among species within the genus; *Gi. gigantea* GGGT 109 performed best among the *Gigaspora* isolates as *S. calospora* CCLS 348 did among the *Scutellispora*.

Single colonization by *Gl. manihot* LMNH 980 or *Rhizobium* increased shoot N concentration by 38% and 17%, respectively. The dual symbiosis increased shoot N concentration by 53%, relative to noninoculated control. *Rhizobium* improved the shoot N concentration of plants with *Gl. manihot* LMNH 980 by

Table 4-27. Shoot and root N concentration of *Stylosanthes guianensis* inoculated with selected VA mycorrhizal fungi and *Rhizobium* (Trial 1).

Treatment	Shoot N conc %	Root N conc %
VAMF + *Rhizobium*:		
Gl. manihot LMNH 980	1.99*a**	1.45 a
Gi. gigantea GGGT 109	1.82 c	1.47 a
Gi. gigantea GMRG 185	1.56 de	1.41 b
Gi. margarita GMRG 444	1.62 d	1.40 b
S. heterogama CHTG 139	1.59 de	1.31 d
S. calospora CCLS 348	1.76 c	1.25 e
S. pellucida CPLC 288	1.61 de	1.34 c
A. scrobiculata ASCB 456	1.89 b	1.32 cd
VAMF + *Rhizobium*:		
Gl. manihot LMNH 980	1.79 c	1.33 cd
Rhizobium	1.52 f	1.44 a
Noninoculated	1.30 g	1.33 cd

* Means represent 12 to 18 replicates.

** Means followed by the same letter are not significantly different at $p \leq 0.05$ by Waller-Duncan K-ratio T test.

11%. Compared with the *Rhizobium* control, VA mycorrhizal fungi did not improve N concentration. In fact, the root N concentration of mycorrhizal plants except those colonized by *Gl. manihot* LMNH 980 and *Gi. gigantea* GGGT 109 was lower than that of their nonmycorrhizal counterparts. The plants did not benefit from single colonization by *Gl. manihot* LMNH 980 but benefited from *Rhizobium* or from the combination of the two symbionts which effected 8% and 9% increases, respectively.

Both shoot and root N content of *S. guianensis* were affected by VA mycorrhiza (Table 4-28). The effect of the symbiosis varied with species and isolates of the fungi. The most significant effect was due to *Gl. manihot* LMNH 980 which increased shoot N content by 2200% and root N content by 1293%. *Gigaspora* species were better than *Scutellispora* species. Among the *Gigaspora* species, *Gi. gigantea* GGGT 109 had the greatest contribution to shoot N content as *Gi. margarita* GMRG 185 had to root N content. *Gigaspora margarita* GMRG 185 was better than GMRG 444 in terms of effect on root N content. Comparing the *Scutellispora* species, *S. heterogama* CHTG 139 and *S. calospora* CCLS 348 were more effective in improving shoot N content than *S. pellucida* CPLC 288. *Acaulospora scrobiculata* ASCB 456 was the least effective among those which successfully colonized *S. guianensis*, but increased shoot N content by 638% and root N content by 321%. Even in the absence of *Rhizobium, Gl. manihot* LMNH 980 increased shoot and root N content by 343%

Table 4-28. Shoot and root total N content of *Stylosanthes guianensis* inoculated with selected VA mycorrhizal fungi and *Rhizobium* (Trial 1).

Treatment	Shoot N content mg	Root N content mg
VAMF + *Rhizobium*:		
Gl. manihot LMNH 980	18.4*a**	8.08 a
Gi. gigantea GGGT 109	14.0 b	6.00 c
Gi. gigantea GMRG 185	12.0 c	6.88 b
Gi. margarita GMRG 444	11.0 c	5.04 d
S. heterogama CHTG 139	8.9 d	3.73 e
S. calospora CCLS 348	8.9 d	3.73 e
S. pellucida CPLC 288	7.2 e	3.75 e
A. scrobiculata ASCB 456	5.9 f	2.44 f
VAMF + *Rhizobium*:		
Gl. manihot LMNH 980	3.1 g	1.36 g
Rhizobium	0.8 h	0.58 h
Noninoculated	0.7 h	0.63 h

* Means represent 12 to 18 replicates.

** Means followed by the same letter are not significantly different at $p \leq 0.05$ by Waller-Duncan K-ratio T test.

and 134%, respectively. *Rhizobium* alone did not affect tissue total N content. However, *Rhizobium* in the presence of *Gl. manihot* LMNH 980 increased N content in shoots or roots by 494%.

Growth response. The most effective isolate of the VA mycorrhizal fungi evaluated was *Gl. manihot* LMNH 980 which increased shoot dry weight by 340% and root dry weight by 1298% (Table 4-29). The effect of isolate LMNH 980 differed from the other VA mycorrhizal fungi. The next effective ones were the *Gigaspora* isolates. *Gigaspora gigantea* GGGT 109 and *Gi. margarita* GMRG 185 were better than GMRG 444. *Scutellispora* species were less effective than the *Gigaspora* species but were more effective than *A. scrobiculata* ASCB 456. The latter increased shoot dry weight and root dry weight by 50% and 365%, respectively relative to *Rhizobium* control. Plants colonized by *S. heterogama* CHTG 139 and *S. calospora* CCLS 348 had greater shoot dry weight than those with *S. pellucida* CPLC 288. *Rhizobium* alone did not affect the shoot and root dry weights, but *Gl. manihot* LMNH 980 alone did by as much as 137%. Together, *Rhizobium* and *Gl. manihot* LMNH 980 stimulated shoot growth by 522% and root growth by 1078%, relative to uninoculated control. Furthermore, *Rhizobium* improved the shoot dry weight of mycorrhizal plants by 162% and root dry weight by 446%.

Correlations. Both nodule number and mycorrhizal colonization were highly correlated with growth variables

Table 4-29. Shoot and root dry weights of *Stylosanthes guianensis* inoculated with selected VA mycorrhizal fungi and *Rhizobium* (Trial 1).

Treatment	Shoot Dry Weight mg		Root Dry Weight mg	
VAMF + *Rhizobium*:				
Gl. manihot LMNH 980	1020*	a**	601	a
Gi. gigantea GGGT 109	850	b	444	c
Gi. margarita GMRG 185	850	bc	531	b
Gi. margarita GMRG 444	740	c	390	d
S. heterogama CHTG 139	620	d	308	e
S. calospora CCLS 348	558	d	323	e
S. pellucida CPLC 288	490	e	302	e
A. scrobiculata ASCB 456	348	f	200	f
VAMF or *Rhizobium*:				
Gl. manihot LMNH 980	389	f	110	g
Rhizobium	232	g	43	h
Noninoculated	164	g	51	h

* Means represent 12 to 18 replicates.

** Means followed by the same letter are not significantly different at $p \leq 0.05$ by Waller-Duncan K-ratio T test.

(Table 4-30). Root mycorrhizal colonization was more correlated to shoot growth than was nodule number. However, the reverse was true in terms of root growth. In regard to N nutrition, both nodule number and mycorrhizal colonization were highly correlated with shoot and root N content but neither was correlated with root N concentration. Mycorrhizal colonization was also more correlated to P nutritional variables than was nodule number. Furthermore, there was a good correlation between nodule number and root VA mycorrhizal colonization.

High correlations of shoot or root dry weight with shoot or root N and P content were found (Table 4-31). However, correlations between shoot or root dry weight and shoot P concentration were low. There was no significant correlation between the growth variables and root P concentration. With respect to correlations between N and P nutrition variables, shoot and root P content were found highly correlated with shoot and root N content (Table 4-32). Shoot P concentration or content was well correlated with shoot N concentration or content. Correlations between root P concentration and N variables were either low or not significant.

Stylosanthes guianensis (Trial 2)

Root VA mycorrhizal colonization. The same isolates of VA mycorrhizal fungi evaluated in Trial 1 were evaluated in Trial 2 except *Gi. margarita* GMRG 185 due to unavailability of

Table 4-30. Pearson coefficients for correlating nodule number and root VAM colonization with various growth and nutritional variables in *Stylosanthes guianensis* (Trial 1).

Variable	Variable	
	Nodule number	Root VAM Colonization***
Shoot fresh weight	0.85*	0.87
Shoot dry weight	0.84	0.87
Root fresh weight	0.87	0.81
Root dry weight	0.83	0.84
Shoot N conc	0.49	0.63
Shoot N content	0.84	0.87
Root N conc	ns**	ns
Root N content	0.83	0.84
Shoot P conc	0.26	0.66
Shoot P content	0.81	0.89
Root P conc	ns	0.27
Root P content	0.78	0.83

* Coefficients are obtained from correlation analysis involving 212 observations per variable.

** ns indicates that correlation is not significant at $p \leq 0.01$.

*** Pearson coefficient for correlating nodule number and root VAM colonization is 0.69.

Table 4-31. Pearson coefficients for correlating shoot and root dry weights with N and P nutrition of *Stylosanthes guianensis* (Trial 1).

Variable	Variable	
	Shoot Dry Weight	Root Dry Weight
Shoot N conc	0.46*	0.45
Shoot N content	0.99	0.95
Root N conc	0.24	0.21
Root N content	0.96	0.99
Shoot P conc	0.39	0.36
Shoot P content	0.96	0.91
Root P conc	ns**	ns
Root P content	0.91	0.95

* Coefficients are obtained from correlation analysis involving 212 observations per variable.

** ns indicates that correlation is not significant at $p \leq 0.01$.

Table 4-32. Pearson coefficients for correlating P nutrition with N nutrition of *Stylosanthes guianensis* (Trial 1).

Variable	Variable			
	Shoot P conc	Shoot P content	Root P conc	Root P content
Shoot N conc	0.59*	0.58	0.18	0.49
Shoot N content	0.42	0.98	ns	0.92
Root N conc	ns**	0.24	0.19	0.29
Root N content	0.36	0.92	ns	0.95

* Coefficients are obtained from correlation analysis involving 212 observations per variable.

** ns indicates that correlation is not significant at $p \leq 0.01$.

spores. The results obtained from the two trials were essentially similar. Plants with *Gl. manihot* LMNH 980 were the most extensively colonized and the level of colonization was different from that due to any other isolate (Table 4-33). *Gigaspora gigantea* GGGT 109 and *Gi. margarita* GMRG 444 colonized the host equally well. Percent colonization by *Gigaspora* isolates was less than that by *Gl. manihot* LMNH 980 but higher than that by *Scutellispora* species. Plants with *S. calospora* CCLS 348 were more colonized than those with *S. pellucida* CPLC 288. The major difference between the results of the two trials was with *A. scrobiculata* ASCB 456; the fungus colonized *S. guianensis* in trial 1 but not in trial 2. *Entrophospora colombiana* ECLB 356, *Gl. etunicatum* LETC 236, LETC 329, and *Gl. mosseae* LMSS 378 consistently failed to colonize the legume in Pacolet soil. *Rhizobium* stimulated root colonization by *Gl. manihot* LMNH 980 by 177%. Control plants remained nonmycorrhizal throughout the duration of the experiment.

Phosphorus nutrition. In the presence of *Rhizobium*, shoot P concentration of *S. guianensis* was not improved by VA mycorrhizal fungi (Table 4-34). However, in the absence of *Rhizobium*, *Gl. manihot* LMNH 980 increased shoot P concentration by 140%. *Rhizobium* did not increase the shoot P concentration of the legume, whether mycorrhizal or not. Plants colonized by *Gl. manihot* LMNH 980 and the *Gigaspora* species had lower shoot P concentration than those colonized

Table 4-33. Root mycorrhizal colonization of *Stylosanthes guianensis* inoculated with selected VA mycorrhizal fungi and *Rhizobium* (Trial 2).

Treatment	Root Mycorrhizal Colonization %
VAMF + *Rhizobium*:	
Gl. manihot LMNH 980	76.5*a**
Gi. gigantea GGGT 109	57.5 b
Gi. margarita GMRG 444	53.4 b
S. heterogama CHTG 139	47.9 c
S. calospora CCLS 348	44.7 c
S. pellucida CPLC 288	35.2 d
E. colombiana ECLB 356	0.0 f
A. scrobiculata ASBC 456	0.0 f
Gl. etunicatum LETC 236	0.0 f
Gl. etunicatum LETC 329	0.0 f
Gl. mosseae LMSS 378	0.0 f
VAMF + *Rhizobium*:	
Gl. manihot LMNH 980	27.6 e
Rhizobium	0.0 f
Noninoculated	0.0 f

* Means represent 6 to 10 replicates.

** Means followed by the same letter are not significantly different at $p \leq 0.05$ by Waller-Duncan K-ratio T test.

Table 4-34. Shoot and root P concentration of *Stylosanthes guianensis* inoculated with selected VA mycorrhizal fungi and *Rhizobium* (Trial 2).

Treatment	Shoot P conc %	Root P conc %
VAMF + *Rhizobium*:		
Gl. manihot LMNH 980	0.032*e**	0.049 c
Gi. gigantea GGGT 109	0.033 e	0.054 b
Gi. margarita GMRG 444	0.024 f	0.046 d
S. heterogama CHTG 139	0.045 c	0.044 d
S. calospora CCLS 348	0.040 d	0.052 c
S. pellucida CPLC 288	0.039 d	0.045 d
VAMF + *Rhizobium*:		
Gl. manihot LMNH 980	0.250 a	Nd***
Rhizobium	0.046 c	0.038 e
Noninoculated	0.104 b	0.091 a

* Means represent 6 to 10 replicates.

** Means followed with the same letter are not significantly different at $p \leq 0.05$ by Waller-Duncan K-ratio T test.

*** Nd indicates no data due to insufficient tissue sample for chemical analysis.

by *Scutellispora* species. Furthermore, the shoot P concentration of noninoculated control plants was higher than that with *Rhizobium*, with or without *Gl. manihot* LMNH 980.

Contrary to the effect on shoot P concentration, VA mycorrhizal fungi increased the root P concentration of rhizobial plants. Those colonized by *Gi. gigantea* GGGT 109 had 42% higher root P concentration than the rhizobial control. The uninoculated plants had higher root P concentration than those with *Rhizobium*, alone or in combination with different VA mycorrhizal fungi.

All Al-tolerant isolates of VA mycorrhizal fungi enhanced shoot and root P content regardless of *Rhizobium* (Table 4-35). Maximum benefit was obtained from *Gl. manihot* LMNH 980 which stimulated shoot P content by 478% in *Rhizobium*-inoculated plants and by 667% in noninoculated ones. Likewise, root P content was enhanced by this isolate by as much as 934% relative to the *Rhizobium* control. *Gigaspora gigantea* GGGT 109 was as effective as *Gl. manihot* LMNH 980 but better than *Gi. margarita* GMRG 444, *S. heterogama* CHTG 139, *S. calospora* CCLS 348 and *S. pellucida* CPLC 288.

Nodulation. Plants colonized by *Gl. manihot* LMNH 980 and *S. heterogama* CHTG 139 were the most nodulated with at least 231% more nodules than the *Rhizobium* control (Table 4-36). Less nodules were formed in plants colonized by *Gi. gigantea* GGGT 109, *Gi. margarita* GMRG 444, *S. calospora* CCLS 348, and *S. pellucida* CPLC 288. Uninoculated control and *Gl. manihot*

Table 4-35. Shoot and root P content of *Stylosanthes guianensis* inoculated with selected VA mycorrhizal fungi and *Rhizobium* (Trial 2).

Treatment	Shoot P content mg	Root P content mg
VAMF + *Rhizobium*:		
Gl. manihot LMNH 980	0.370*b**	0.362 a
Gi. gigantea GGGT 109	0.315 c	0.332 a
Gi. margarita GMRG 444	0.168 d	0.168 bc
S. heterogama CHTG 139	0.271 c	0.203 b
S. calospora CCLS 348	0.209 d	0.178 b
S. pellucida CPLC 288	0.196 d	0.142 c
VAMF or *Rhizobium*:		
Gl. manihot LMNH 980	0.445 a	Nd***
Rhizobium	0.064 e	0.035 d
Noninoculated	0.058 e	0.032 d

* Means represent 6 to 10 replicates.

** Means followed by the same letter are not significantly different at $p \leq 0.05$ by Waller-Duncan K-ratio T test.

*** Nd indicates no data due to insufficient tissue sample for chemical analysis.

Table 4-36. Nodule number of *Stylosanthes guianensis* inoculated with selected VA mycorrhizal fungi and *Rhizobium* (Trial 2).

Treatment	Nodule Number
VAMF + *Rhizobium*:	
Gl. manihot LMNH 980	46*a**
Gi gigantea GGGT 109	34 b
Gi. margarita GMRG 444	30 b
S. heterogama CHTG 139	43 a
S. calospora CCLS 348	32 b
S. pellucida CPLC 288	31 b
VAMF or *Rhizobium*:	
Gl. manihot LMNH 980	2 d
Rhizobium	13 c
Noninoculated	1 d

* Means represent 6 to 10 replicates.

** Means followed by the same letter are not significantly different at $p<0.05$ by Waller-Duncan K-ratio T test.

LMNH 980 control seedlings developed few nodules. *Rhizobium* inoculation improved nodulation by 1200% in nonmycorrhizal plants, and by 2200% in mycorrhizal ones.

Nitrogen nutrition. In contrast to the results obtained from trial 1, VA mycorrhizal fungi did not increase the shoot N concentration of *S. guianensis* in trial 2 (Table 4-37). In fact, the shoot N concentration of mycorrhizal plants was lower than that of the corresponding *Rhizobium* control except those plants with *S. heterogama* CHTG 139. However, *Gl. manihot* LMNH 980 alone increased shoot N concentration by 47% relative to uninoculated plants. *Rhizobium* increased shoot N concentration of both mycorrhizal and nonmycorrhizal plants by 11% and 76%, respectively.

The root N status of *Gl. manihot* LMNH 980 control plants was not determined due to insufficient tissue sample for chemical analysis. The root N concentration of mycorrhizal plants was higher than their nonmycorrhizal counterparts except those colonized by *S. heterogama* CHTG 139 and *S. pellucida* CPLC 288. Plants with *Gi. margarita* GMRG 444 had the highest root N concentration, 14% more than *Rhizobium* control. Furthermore, *Rhizobium* increased root N concentration by 17%.

All VA mycorrhizal fungi increased shoot and root total N content (Table 4-38). The greatest increases equivalent to 682% and 744%, respectively over the *Rhizobium* control, were due to *Gl. manihot* LMNH 980. *Gigaspora* species were generally

Table 4-37. Shoot and root N concentration of *Stylosanthes guianensis* inoculated with selected VA mycorrhizal fungi and *Rhizobium* (Trial 2).

Treatment	Shoot N conc %	Root N conc %
VAMF + *Rhizobium*:		
Gl. manihot LMNH 980	1.47*bc**	1.28 cd
Gi. gigantea GGGT 109	1.42 bc	1.32 b
Gi. margarita GMRG 444	1.49 b	1.39 a
S. heterogama CHTG 139	1.57 a	1.21 f
S. calospora CCLS 348	1.48 bc	1.30 bc
S. pellucida CPLC 288	1.40 c	1.25 de
VAMF + *Rhizobium*:		
Gl. manihot LMNH 980	1.32 d	Nd***
Rhizobium	1.58 a	1.22 ef
Noninoculated	0.90 e	1.04 g

* Means represent 6 to 10 replicates.

** Means followed by the same letter are not significantly different at $p \leq 0.05$ by Waller-Duncan K-ratio T test.

*** Nd indicates not determined due to insufficient sample for chemical analysis.

Table 4-38. Shoot and root N content of *Stylosanthes guianensis* inoculated with selected VA mycorrhizal fungi and *Rhizobium* (Trial 2).

Treatment	Shoot N content mg	Root N content mg
VAMF + *Rhizobium*:		
Gl. manihot LMNH 980	17.2*a**	9.37 a
Gi. gigantea GGGT 109	13.3 b	7.96 b
Gi. margarita GMRG 444	10.4 c	5.08 cd
S. heterogama CHTG 139	9.5 c	5.70 c
S. calospora CCLS 348	7.6 d	4.47 de
S. pellucida CPLC 288	7.0 d	3.94 e
VAMF + *Rhizobium*:		
Gl. manihot LMNH 980	2.4 e	Nd***
Rhizobium	2.2 e	1.11 f
Noninoculated	0.5 f	0.37 f

* Means represent 6 to 10 replicates.

** Means followed by the same letter are not significantly different at $p \leq 0.05$ by Waller-Duncan K-ratio T test.

*** Nd indicates no data due to insufficient tissue sample for chemical analysis.

more effective than *Scutellispora* species. Significant differences within a genus were also observed. Single symbiosis with *Gl. manihot* LMNH 980 or *Rhizobium* stimulated shoot N content by at least 340% while dual symbiosis caused a 3340% increase relative to uninoculated control. Likewise, root total N content was increased 200% by either symbiont and 432% by both.

Growth response. As in trial 1, the best response to inoculation with VA mycorrhizal fungi was obtained from *Gl. manihot* LMNH 980 which stimulated shoot growth by 1149% and root growth by 713% (Table 4-39). This was then followed by *Gigaspora* species. *Gigaspora gigantea* GGGT 109 was more effective than *Gi. margarita* GMRG 444. Among the VA mycorrhizal fungi which were able to colonize the host, *S. calospora* CCLS 348 and *S. pellucida* CPLC 288 were the least effective in stimulating plant growth, but nevertheless improved shoot weight by at least 260% and root weight by at least 251%.

Rhizobium alone did not affect the growth of *S. guianensis* but mycorrhiza alone increased shoot growth by 215% and root growth by 250%. Together the two symbionts improved shoot growth by 2001% and root growth by 1997%, relative to uninoculated seedlings. Although there was no growth response to *Rhizobium* in the absence of mycorrhizal fungi, it stimulated shoot and root growth of mycorrhizal plants by 568% and 499%, respectively.

Table 4-39. Shoot and root dry weights of *Stylosanthes guianensis* inoculated with selected VA mycorrhizal fungi (Trial 2).

Treatment	Shoot Dry weight mg		Root Dry weight mg	
VAMF + *Rhizobium*:				
Gl. manihot LMNH 980	1303*	a**	797	a
Gi. gigantea GGGT 109	1030	b	662	b
Gi. margarita GMRG 444	765	c	398	d
S. heterogama CHTG 139	666	cd	511	c
S. calospora CCLS 348	570	de	375	d
S. pellucida CPLC 288	554	e	344	d
VAMF + *Rhizobium*:				
Gl. manihot LMNH 980	195	f	133	e
Rhizobium	154	fg	98	ef
Noninoculated	62	g	38	f

* Means represent 6 to 10 replicates.

** Means with the same letter are not significantly different at $p \leq 0.05$ by Waller-Duncan K-ratio T test.

Correlations. Mycorrhizal colonization was more correlated to growth variables than was nodule number (Table 4-40). This was not clearly observed in trial 1. Both nodule number and root mycorrhizal colonization were weakly correlated with N concentration but highly correlated with total N content, consistent with the results of trial 1. Nodule number was negatively correlated with P concentration. Similarly, the correlation between mycorrhizal colonization and P concentration was either negative or not significant. As in trial 1, nodule number and root VA mycorrhizal colonization were both highly correlated with root P content.

Both shoot and root dry weights were highly correlated with N content, fairly correlated with root N concentration, and not correlated with shoot N concentration (Table 4-41). Growth variables were negatively correlated with shoot P concentration and not correlated with root P concentration. On the other hand, growth was fairly correlated with shoot P content and highly correlated with root P content.

There were high negative correlations between shoot P and root N concentration, between shoot P concentration and root N content, and between root P and shoot N concentration (Table 4-42). On the contrary, P content was usually highly correlated with N content.

Table 4-40. Pearson coefficients for correlating nodule number and root VAM colonization with various growth and nutritional variables in *Stylosanthes guianensis* (Trial 2).

Variable	Variable	
	Nodule Number	Root VAM Colonization***
Shoot fresh weight	0.76*	0.91
Shoot dry weight	0.76	0.91
Root fresh weight	0.78	0.89
Root dry weight	0.80	0.90
Shoot N conc	0.37	ns**
Shoot N content	0.77	0.91
Root N conc	0.49	0.55
Root N content	0.79	0.92
Shoot P conc	-0.60	-0.29
Shoot P content	0.35	0.68
Root P conc	-0.33	ns
Root P content	0.75	0.89

* Coefficients are obtained from correlation analysis involving 94 to 101 observations per variable.

** ns indicates that correlation is not significant at $p \leq .01$.

*** Coefficient for correlating nodule number with root VAM colonization is 0.71.

Table 4-41. Pearson coefficients for correlating shoot and root dry weights with N and P nutrition of *Stylosanthes guianensis* (Trial 2).

Variable	Variable	
	Shoot Dry Weight	Root Dry Weight
Shoot N conc	ns**	ns
Shoot N content	0.99*	0.96
Root N conc	0.53	0.47
Root N content	0.96	0.99
Shoot P conc	-0.45	-0.44
Shoot P content	0.64	0.63
Root P conc	ns	ns
Root P content	0.95	0.98

* Coefficients are obtained from correlation analysis involving 94 to 101 observations per variable.

** ns indicates that correlation is not significant at $p \leq 0.01$.

Table 4-42. Pearson coefficients for correlating P nutrition with N nutrition of *Stylosanthes guianensis* (Trial 2).

Variable	Variable			
	Shoot P conc	Shoot P content	Root P conc	Root P content
Shoot N conc	-0.33	ns	-0.86	ns
Shoot N content	0.46	0.63	ns	0.93
Root N conc	-0.78	0.42	-0.55	0.42
Root N content	-0.70	0.92	ns	0.98

* Coefficients are obtained from correlation analysis involving 94 to 101 observations per variable.

** ns indicates that correlation is not significant at $p \leq 0.01$.

Leucaena leucocephala

Root VA mycorrhizal colonization and nodulation. *Glomus manihot* LMNH 980 colonized *L. leucocephala* grown in a high-Al acid soil. The extent of colonization was higher in the presence than in the absence of *Rhizobium* (Figure 4-1). As to the effect on nodulation, *Rhizobium* increased the number of nodules in both mycorrhizal and nonmycorrhizal plants, but increased the total nodule weight only in mycorrhizal ones *Glomus manihot* LMNH 980 increased the number and weight of nodules regardless of *Rhizobium*.

Phosphorus nutrition. The VA mycorrhizal fungus increased the shoot and root P concentration regardless of *Rhizobium* (Figure 4-2). The magnitude of increase is greater in the absence than in the presence of *Rhizobium*; 317% increase in shoot and 225% in root P concentrations were attained. *Rhizobium* increased root but not shoot P concentration.

Similarly, *Gl. manihot* LMNH 980 increased shoot or root total P content with or without *Rhizobium* (Figure 4-3). Shoot P content was increased by 685% in rhizobial plants and by 747% in nonrhizobial ones while root P content was increased by 796% and 541%, respectively. The P content of plants inoculated with both *Gl. manihot* LMNH 980 and *Rhizobium* was greater than those inoculated with either symbiotic partner.

Nitrogen nutrition. Relative to uninoculated control, *Gl. manihot* LMNH 980 improved shoot N concentration by 26%,

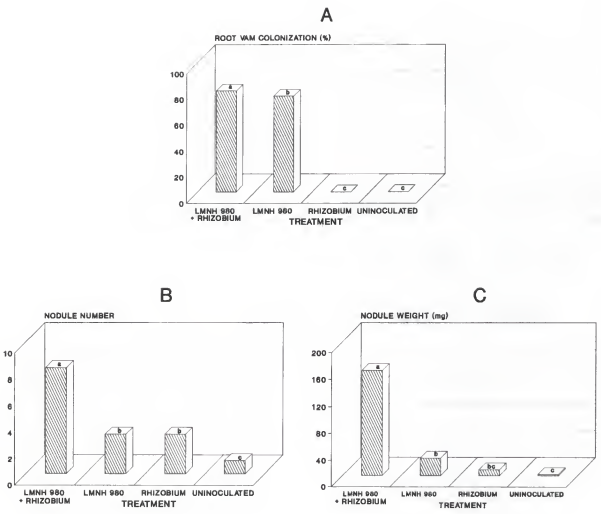

Figure 4-1. Root VA mycorrhizal colonization and nodulation of *Leucaena leucocephala* inoculated with *Glomus manihot* LMNH 980 and *Rhizobium*. A. Root VA mycorrhizal colonization. B. Nodule number. C. Nodule Weight. Means represent 24 replicates. Means with the same letter are not significantly different at $p \leq 0.05$ by Waller-Duncan K-ratio T test.

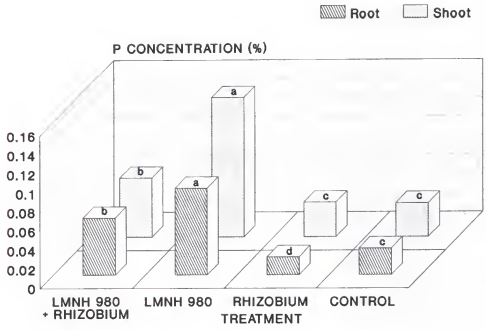

Figure 4-2. Shoot and root P concentration of *Leucaena leucocephala* inoculated with *Glomus manihot* LMNH 980 or *Rhizobium*. Means represent 24 replicates. Means with the same letter are not significantly different at $p \leq 0.05$ by Waller-Duncan K-ratio T test.

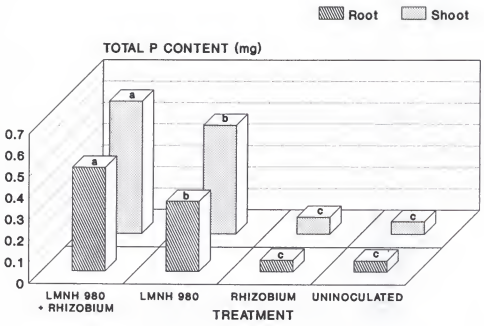

Figure 4-3. Shoot and root total P content of *Leucaena leucocephala* inoculated with *Glomus manihot* LMNH 980 or *Rhizobium*. Means represent 24 replicates. Means with the same letter are not significantly different at $p \leq 0.05$ by Waller-Duncan K-ratio T test.

and *Rhizobium* by 32% (Figure 4-4). Shoot N concentration of plants with double symbiosis was not better than those plants with either *Gl. manihot* LMNH 980 or *Rhizobium*.

Rhizobium alone increased root N concentration by 21% whereas, *Gl. manihot* LMNH 980 alone did not. Again, plants with both organisms were not better than those with *Rhizobium* alone or with the mycorrhizal fungus alone.

The shoot and root total N content of *L. leucocephala* were affected by *Gl. manihot* LMNH 980 or *Rhizobium* (Figure 4-5). The VA mycorrhizal fungus improved shoot total N of *Rhizobium*-inoculated plants by 345% while *Rhizobium* improved the shoot total N of mycorrhizal plants by 192%. *Glomus manihot* LMNH 980 increased shoot total N by 154%, *Rhizobium* by 66%, and together by 640% relative to uninoculated control. All treatment means were significantly different from one another.

In terms of total N in roots, the greatest effect was due to *Gl. manihot* LMNH 980 + *Rhizobium* where the fungus effected a 142% increase while the latter caused a 139% increase. As compared with the noninoculated plants, those colonized separately by either *Gl. manihot* LMNH 980 or *Rhizobium* had at least 91% greater root N concentration while those colonized by both had 362% greater.

Growth response. *Rhizobium* increased shoot and root fresh and dry weights of mycorrhizal seedlings, but increased only the root weights of nonmycorrhizal ones. On the other

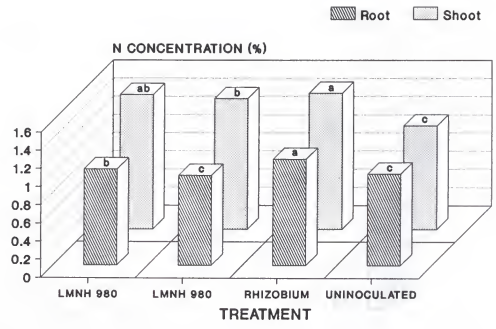

Figure 4-4. Shoot and root N concentration of *Leucaena leucocephala* inoculated with *Glomus manihot* LMNH 980 or *Rhizobium*. Means represent 24 replicates. Means with the same letter are not significantly different at $p \leq 0.05$ by Waller-Duncan K-ratio T test.

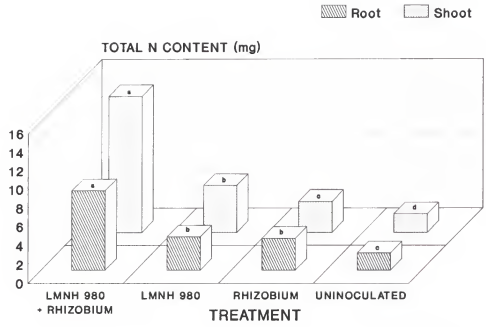

Figure 4-5. Shoot and root total N content of *Leucaena leucocephala* inoculated with *Glomus manihot* LMNH 980 and *Rhizobium*. Means represent 24 replicates. Means with the same letter are not significantly different at $p \leq 0.05$ by Waller-Duncan K-ratio T test.

hand, *Gl. manihot* LMNH 980 increased shoot and root fresh and dry weights, either alone or in combination with *Rhizobium* (Figure 4-6). There were also growth responses to separate inoculations with *Rhizobium* or *Gl. manihot* LMNH 980. However, a far more significant response was obtained when both symbionts were inoculated together.

Plants colonized by both organisms were the tallest and had the largest diameter where the mycorrhizal fungus effected a 60% increase in height and 69% increase in diameter while *Rhizobium* had caused 31% and 50% increases in height and diameter, respectively (Figure 4-7). In regard to the effect of single symbiosis, *Gl. manihot* LMNH 980 proved effective and stimulated height and diameter growth whereas, *Rhizobium* did not.

Correlations. There were significant positive correlations between nodulation or mycorrhizal colonization on one hand, and growth or N and P nutrition on the other hand (Table 4-43). Mycorrhizal colonization was highly correlated with shoot or root P concentration and content. The P status of the plant particularly in terms of content was fairly correlated with nodule weight or nodule number, which in turn was fairly correlated with root N content, as well as with all the growth parameters evaluated. Total nodule weight was more correlated with growth variables than was nodule number. Root mycorrhizal colonization was fairly correlated with either nodule number or nodule weight.

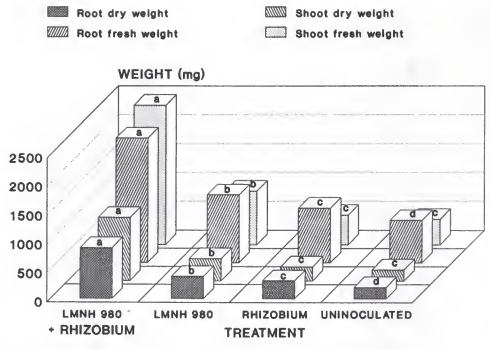

Figure 4-6. Shoot and root fresh and dry weights of *Leucaena leucocephala* inoculated with *Glomus manihot* LMNH 980 and *Rhizobium*. Means represent 24 replicates. Means with the same letter are not significantly different at $p \leq 0.05$ by Waller-Duncan K-ratio T test.

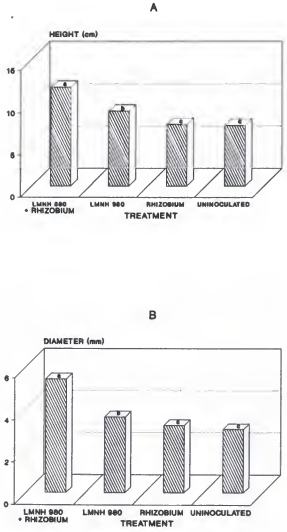

Figure 4-7. Height and diameter of *Leucaena leucocephala* inoculated with *Glomus manihot* LMNH 980 and *Rhizobium*. A. Height. B. Diameter. Means represent 24 replicates. Means with the same letter are not significantly different at $p \leq 0.05$ by Waller-Duncan K-ratio T test.

Table 4-43. Pearson coefficients for correlating nodulation and root VAM colonization with various growth and nutrition variables in *Leucaena leucocephala*.

Variable	Variable		
	Nodule Number	Nodule Weight	Root VAM Colonization
Height	0.61*	0.73	0.78
Diameter	0.62	0.68	0.68
Root Fresh Weight	0.64	0.74	0.72
Root Dry Weight	0.71	0.79	0.71
Shoot N Content	0.37	0.28	0.40
Shoot Total N Uptake	0.65	0.73	0.72
Root N Content	ns**	ns	-0.40
Root Total N Uptake	0.72	0.79	0.67
Shoot P Content	ns	ns	0.69
Shoot Total P Uptake	0.54	0.60	0.91
Root P Content	ns	0.29	0.85
Root Total P Uptake	0.63	0.72	0.90

* Coefficients are obtained from correlation analysis involving 96 observations per variable.

** ns indicates that correlation is not significant at $p \leq 0.01$.

*** Pearson coefficients for correlating nodule number and nodule weight with root VAM colonization are 0.73 and 0.84, respectively.

Both shoot and root dry weights were highly correlated with N and P contents (Table 4-44). However, the correlation between shoot and root dry weights with N or P concentration were either weak or not significant.

Phosphorus content was highly correlated with N content and weakly correlated with shoot N concentration (Table 4-45). Shoot or root P concentration was negatively or not significantly correlated with N concentration.

Centrosema pubescens

Root VA mycorrhizal colonization and nodulation. *Glomus manihot* LMNH 980 colonized *C. pubescens* grown in a high-Al acid soil quite extensively (Figure 4-8). *Rhizobium* inoculation enhanced mycorrhizal colonization by 57%. *Rhizobium* and uninoculated control seedlings remained nonmycorrhizal. *Rhizobium* failed to nodulate *C. pubescens* in the absence of *Gl. manihot* LMNH 980. The VA mycorrhizal fungus favored nodulation. The mycorrhizal control seedlings developed few nodules from indigenous *Rhizobium*.

Phosphorus nutrition. The VA mycorrhizal fungus had a significant effect on shoot and root P concentration of *C. pubescens* where increases of as much as 620% and 314%, respectively, were obtained (Figure 4-9). The shoot P concentration of mycorrhizal plants was not affected by *Rhizobium* while root P concentration was increased by 14%.

Table 4-44. Pearson coefficients for correlating shoot and root dry weights with N and P nutrition of *Leucaena leucocephala*.

Variable	Variable	
	Shoot Dry Weight	Root Dry Weight
Shoot N Conc	0.34*	0.48
Shoot N Content	0.98	0.94
Root N Conc	ns	ns
Root N Content	0.91	0.99
Shoot P Conc	ns	ns
Shoot P Content	0.80	0.73
Root P Conc	0.35	0.34
Root P Content	0.82	0.88

* Coefficients are obtained from correlation analysis involving 96 observations per variable.

** ns indicates that correlation is not significant at $p \leq 0.01$.

Table 4-45. Pearson coefficients for correlating P nutrition with N nutrition in *Leucaena leucocephala*.

Variable	Variable			
	Shoot P Conc	Shoot P Content	Root P Conc	Root P Content
Shoot N Conc	ns**	0.35	ns	0.41
Shoot N Content	ns	0.78	0.34	0.83
Root N Conc	-0.48	-0.29	-0.55	-0.29
Root N Content	ns	0.71	0.29	0.85

* Coefficients are obtained from correlation analysis involving 96 observations per variable.

** ns indicates that correlation is not significant at $p \leq 0.01$.

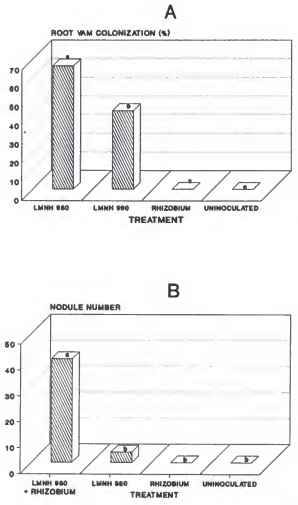

Figure 4-8. Root VA mycorrhizal colonization and nodulation of *Centrosema pubescens* inoculated with *Glomus manihot* LMNH 980 and *Rhizobium*. A. Root VA mycorrhizal colonization. B. Nodule number. Means represent 12 replicates. Means with the same letter are not significantly different at $p \leq 0.05$ by Waller-Duncan K-ratio T test.

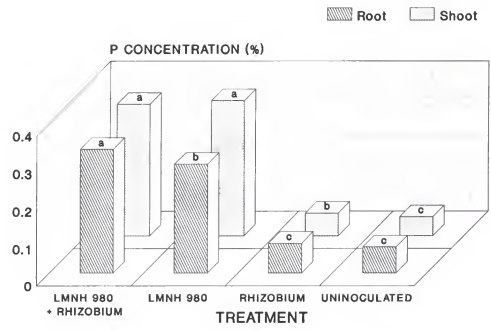

Figure 4-9. Shoot and root P concentration of *Centrosema pubescens* inoculated with *Glomus manihot* LMNH 980 and *Rhizobium*. Means represent 12 replicates. Means with the same letter are not significantly different at $p \leq 0.05$ by Waller-Duncan K-ratio T test.

The greatest amount of P was taken up by plants with both *Gl. manihot* LMNH 980 and *Rhizobium* (Figure 4-10). The double symbioses caused an increase of 1101% in shoot P content and 929% in root P content over the uninoculated control, and an increase of 19% over *Gl. manihot* LMNH 980 only.

Nitrogen nutrition. The shoot and root N concentration of mycorrhizal plants were lower than nonmycorrhizal ones, whether the fungus was inoculated alone or in combination with *Rhizobium* (Figure 4-11). Furthermore, *Rhizobium* had no significant effect on root N concentration of either mycorrhizal or nonmycorrhizal plants.

Glomus manihot increased the shoot and root total N content of *C. pubescens* by 34% and 88%, respectively (Figure 4-12). On the other hand, *Rhizobium* did not affect the N nutrition of the legume at all. Yet, greater improvements were obtained when the two symbionts were inoculated together.

Growth response. Shoot and root fresh and dry weights were increased by *Gl. manihot* LMNH 980, but not by *Rhizobium* (Figure 4-13). Similar effects of the symbionts on shoot length, number of leaves, and number of internodes were observed (Figure 4-14).

Correlations. Compared with nodule number, root mycorrhizal colonization was more correlated with plant growth, N nutrition, and P nutrition (Table 4-46). Significant high correlations were obtained between root colonization and all P variables. The P status of the plant

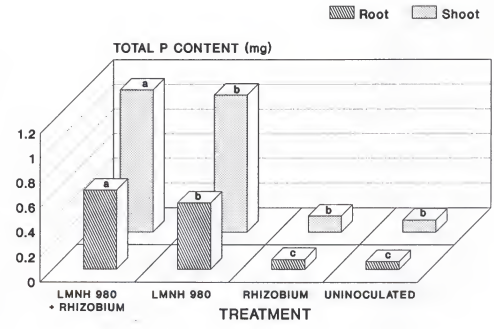

Figure 4-10. Shoot and root total P content of *Centrosema pubescens* inoculated with *Glomus manihot* LMNH 980 and *Rhizobium*. Means represent 12 replicates. Means with the same letter are not significantly different at $p \leq 0.05$ by Waller-Duncan K-ratio T test.

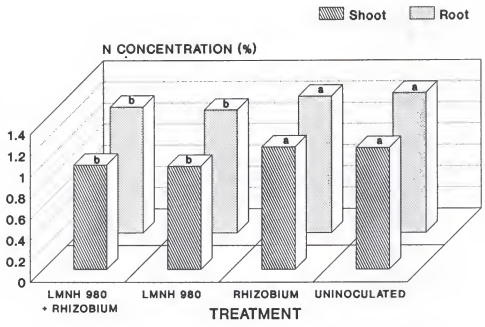

Figure 4-11. Shoot and root N concentration of *Centrosema pubescens* inoculated with *Glomus manihot* LMNH 980 and *Rhizobium*. Means represent 12 replicates. Means with the same letter are not significantly different at $p \leq 0.05$ by Waller-Duncan K-ratio T test.

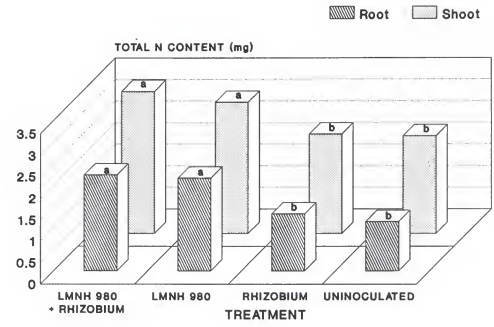

Figure 4-12. Shoot and root N content of *Centrosema pubescens* inoculated with selected VA mycorrhizal fungi and *Rhizobium*. Means represent 12 replicates. Means with the same letter are not significantly different at $p \leq 0.05$ by Waller-Duncan K-ratio T test.

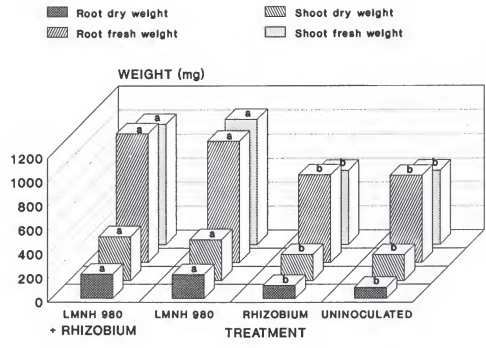

Figure 4-13. Shoot and root fresh and dry weights of *Centrosema pubescens* inoculated with selected VA mycorrhizal fungi and *Rhizobium*. Means represent 12 replicates. Means with the same letter are not significantly different at $p \leq 0.05$ by Waller-Duncan K-ratio T test.

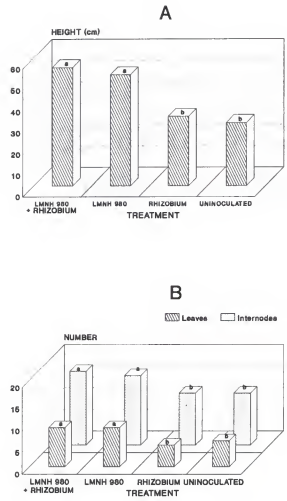

Figure 4-14. Shoot length, number of leaves, and number of internodes of *Centrosema pubescens* inoculated with selected VA mycorrhizal fungi and *Rhizobium*. A. Height. B. Number of leaves and internodes. Means represent 12 replicates. Means with the same letter are not significantly different at $p \leq 0.05$ by Waller-Duncan K-ratio T test.

Table 4-46. Pearson coefficients for correlating nodulation and root VAM colonization with various growth and nutrition variables in *Centrosema pubescens*.

	Variable	
Variable	Nodule number	Root VAM Colonization***
Height	0.39*	0.69
Number of Leaves	0.39	0.71
Number of Internodes	0.46	0.70
Shoot fresh weight	0.40	0.80
Shoot dry weight	0.51	0.84
Root fresh weight	0.45	0.76
Root dry weight	0.43	0.73
Shoot N content	ns**	-0.74
Shoot total N uptake	0.47	0.72
Root N content	ns	-0.65
Root total N uptake	0.43	0.71
Shoot P content	0.49	0.92
Shoot total P uptake	0.51	0.93
Root P content	0.60	0.95
Root total P uptake	0.57	0.90

* Coefficients are obtained from correlation analysis involving 48 observations per variable.

** ns indicates that correlation is not significant at $p \leq 0.01$.

*** Pearson coefficient for correlating nodule number and root VAM colonization is 0.69.

was well correlated with nodule number, which in turn was well correlated with shoot and root total N content as well as with all the growth variables.

Shoot and root dry weights were negatively correlated with N concentration but positively correlated with N content (Table 4-47). With respect to P, plant dry weight was highly correlated with both concentration and content. Correlations between shoot dry weight and shoot total N or P and between root dry weight and root total N or P were noticeably very high.

Shoot or root N concentration was negatively correlated with all P variables while shoot or root N content was positively correlated with all P variables (Table 4-48). Moreover, shoot or root P concentration was highly correlated with shoot or root total N content.

Effectiveness of VA Mycorrhizal Fungi in Relation to Al Tolerance

Root VA mycorrhizal colonization, N and P content, nodulation, and plant growth response of *P. phaseoloides* (Table 4-49) and *S. guianensis* (Table 4-50) were all highly correlated with spore germination, by Pearson product-moment correlation analysis. The same plant parameters were not correlated with either hyphal length or mycelial growth index by the same analysis (data not shown) but were found correlated by Spearman rank correlation (Tables 4-51 and 4-52).

Table 4-47. Pearson coefficients for correlating shoot and root dry weights with N and P nutrition *Centrosema pubescens*.

Variable	Variable	
	Shoot dry weight	Root dry weight
Shoot N conc	-0.68*	-0.65
Shoot N content	0.96	0.76
Root N conc	-0.65	-0.66
Root N content	0.81	0.99
Shoot P conc	0.83	0.80
Shoot P content	0.91	0.82
Root P conc	0.83	0.78
Root P content	0.84	0.91

* Coefficients are obtained from correlation analysis involving 48 observations per variable.

** ns indicates that correlation is not significant at $p<0.01$.

Table 4-48. Pearson coefficients for correlating P nutrition with N nutrition in *Centrosema pubescens*.

Variable	Variable			
	Shoot P Conc	Shoot P Content	Root P Conc	Root P Content
Shoot N Conc	-0.79*	-0.78	-0.78	-0.75
Shoot N Content	0.69	0.78	0.70	0.72
Root N Conc	-0.75	-0.74	-0.73	-0.72
Root N Content	0.75	0.78	0.74	0.88

* Coefficients are obtained from correlation analysis involving 48 observations per variable.

** ns indicates that correlation is not significant at $p<0.01$.

Table 4-49. Pearson coefficients for correlating percent spore germination with growth and nutritional variables of *Pueraria phaseoloides* in a 100% Al-saturated soil.

	Spore Germination[#]	
Variable	Trial 1	Trial 2
Trial 1:		
Root VAM Colonization[#]	0.92 **	0.87 **
Shoot P Content	0.72 *	0.69 *
Root P Content	0.79 *	0.77 *
Shoot N Content	0.77 *	0.75 *
Root N Content	0.78 *	0.76 *
Nodule Number	0.74 *	0.71 *
Nodule Weight	0.79 *	0.81 **
Shoot Dry Weight	0.81 **	0.81 **
Root Dry Weight	0.78 *	0.76 *
Trial 2:		
Root VAM Colonization[#]	0.86 **	0.85 **
Shoot P Content	0.72 *	0.70 *
Root P Content	0.71 *	0.72 *
Shoot N Content	0.74 *	0.73 *
Root N Content	ns	ns
Nodule Number	ns	ns
Nodule Weight	ns	ns
Shoot Dry Weight	0.72 *	0.72 *
Root Dry Weight	ns	ns

Arcsine-transformed values were used in correlation analysis.

ns= not significant at $p<0.05$; *= significant at $p<0.05$; **= significant at $p<0.01$.

Table 4-50. Pearson coefficients for correlating percent spore germination with growth and nutritional variables of *Stylosanthes guianensis* in a 100% Al-saturated soil.

	Spore Germination#	
Variables	Trial 1	Trial 2
Trial 1:		
Root VAM Colonization#	0.95 **	0.92 **
Shoot P Content	0.85 **	0.81 **
Root P Content	0.91 **	0.83 **
Shoot N Content	0.90 **	0.87 **
Root N Content	0.91 **	0.85 **
Nodule Number	0.80 **	0.71 *
Shoot Dry Weight	0.89 **	0.84 **
Root Dry Weight	0.91 **	0.84 **
Trial 2:		
Root VAM Colonization#	0.91 **	0.87 **
Shoot P Content	0.83 **	0.77 *
Root P Content	0.83 **	0.81 *
Shoot N Content	0.89 **	0.88 **
Root N Content	0.85 **	0.83 **
Nodule Number	0.78 *	ns
Shoot Dry Weight	0.90 **	0.90 **
Root Dry Weight	0.85 **	0.81 *

Arcsine-transformed values were used in correlation analysis.

ns= not significant at $p \leq 0.05$; *= significant at $p \leq 0.05$; **= significant at $p \leq 0.01$.

Table 4-51. Spearman coefficients for correlating hyphal length and mycelial growth index (MGI) of VA mycorrhizal fungi with growth and nutritional variables of *Pueraria phaseoloides* in a 100% Al-saturated soil.

	Variable			
	Trial 1		Trial 2	
Variable	Hyphal Length	MGI	Hyphal Length	MGI
Trial 1:				
Root VAM Colonization#	0.66 *	0.75 *	0.70 *	0.87 **
Shoot P Content	ns	0.71 *	ns	0.68 *
Root P Content	ns	0.73 *	0.69 *	0.85 **
Shoot N Content	0.68 *	0.76 *	ns	0.73 *
Root N Content	ns	0.72 *	0.69 *	0.84 **
Nodule Number	0.83 **	0.86 **	0.90 **	0.88 **
Nodule Weight	0.73 *	0.81 **	0.71 *	0.88 **
Shoot Dry Weight	0.71 *	0.79 **	0.73 *	0.91 **
Root Dry Weight	ns	0.71 *	0.68 *	0.83 **
Trial 2:				
Root VAM Colonization#	ns	ns	ns	0.83 **
Shoot P Content	ns	0.78 *	0.72 *	0.88 **
Root P Content	ns	0.72 *	ns	0.89 **
Shoot N Content	ns	ns	ns	0.86 **
Root N Content	ns	ns	ns	0.86 **
Nodule Number	ns	ns	ns	0.74 *
Nodule Weight	ns	ns	ns	0.76 *
Shoot Dry Weight	ns	ns	ns	0.86 **
Root Dry Weight	ns	ns	ns	0.86 **

Correlation analysis involving percent root VAM colonization was done with arcsine-transformed values.

ns= not significant at $p \leq 0.05$; *= significant at $p \leq 0.05$; **= significant at $p \leq 0.01$.

Table 4-52. Spearman coefficients for correlating hyphal length and mycelial growth index (MGI) of VA mycorrhizal fungi with growth and nutritional variables of *Stylosanthes guianensis* in a 100% Al-saturated soil.

	Variable			
	Trial 1		Trial 2	
Variable	Hyphal Length	MGI	Hyphal Length	MGI
Trial 1:				
Root VAM Colonization#	ns	0.70 *	0.68 *	0.93 **
Shoot P Content	0.69 *	0.78 *	0.73 *	0.90 **
Root P Content	ns	ns	ns	0.76 *
Shoot N Content	0.68 *	0.76 *	0.72 *	0.91 **
Root N Content	ns	ns	ns	0.81 **
Nodule Number	ns	ns	ns	0.73 *
Shoot Dry Weight	0.67 *	0.76 *	0.71 *	0.87 **
Root Dry Weight	ns	0.68 *	0.68 *	0.86 **
Trial 2:				
Root VAM Colonization#	0.76 *	0.86 **	0.76 *	0.90 **
Shoot P Content	ns	ns	ns	ns
Root P Content	ns	0.76 *	ns	0.78 *
Shoot N Content	0.75 *	0.85 **	0.75 *	0.90 **
Root N Content	0.73 *	0.83 *	0.73 *	0.83 *
Nodule Number	ns	ns	ns	ns
Shoot Dry Weight	0.75 *	0.85 **	0.75 *	0.90 **
Root Dry Weight	0.73 *	0.83 *	0.73 *	0.83 *

Correlation analysis involving percent root VAM colonization was done with arcsine-transformed values.

ns= not significant at $p \leq 0.05$; *= significant at $p \leq 0.05$; **= significant at $p \leq 0.01$.

Discussion

That VA mycorrhizal fungi improve P nutrition of tropical forage legumes has been demonstrated previously (Salinas et al., 1985; Habte and Manjunath, 1987; Medina et al., 1988; Medina et al., 1990). The present study aimed to determine the relationship of the degree of Al tolerance of these fungi with the level of root VA mycorrhizal colonization produced in a high-Al acid soil and, consequently, with the effectiveness of these fungi in improving P nutrition of host legumes in such soil. Since P is important for nodulation and effective N_2 fixation, N nutrition of the legumes in this soil may also be related to Al tolerance.

The symbiotic VA mycorrhizal association was successfully established in the forage legumes, *P. phaseoloides, S. guianensis, L. leucocephala, and C. pubescens* grown in a P-deficient, high-Al, acid mineral soil. In the first two hosts where several species and isolates of VA mycorrhizal fungi were evaluated, the success of establishing the symbiosis depended on the ability of the isolate to tolerate high Al. *Glomus etunicatum* LETC 236, LETC 329, LETC 455 and *Gl. mosseae* LMSS 378 which were found very sensitive to soil acidity and Al (Chapter III) failed to colonize the legumes in repeated experiments. Other studies have also shown that root colonization by *Glomus macrocarpum* (Graw, 1979) and by *Gl.*

mosseae (Siqueira et al., 1984) was adversely affected by soil acidity and Al.

Aluminum-tolerant isolates, *Gl. manihot* LMNH 980, *Gi. gigantea* GGGT 109 and GGGT 663, *Gi. margarita* GMRG 185 and GMRG 444, *S. heterogama* CHTG 139, *S. calospora* CCLS 348, and *S. pellucida* CPLC 288, consistently colonized *P. phaseoloides* and *S. guianensis*. Just as tolerance to soil acidity and Al varied with genera and species of VA mycorrhizal fungi and often, with isolates of the same species (Chapter III), so did the level of root VA mycorrhizal colonization in this study. A similar intraspecific variation in root colonization by *Gigaspora gigantea* at low soil pH was reported by Lambert and Cole (1980). Across all isolates, percent mycorrhizal colonization was highly correlated with spore germination, hyphal length, and mycelial growth index obtained from the Al tolerance assay in Pacolet soil.

Glomus manihot LMNH 980 colonized the host most extensively. This fungus was originally isolated from Pacolet sandy clay loam and was expected to show high tolerance to soil acidity and Al. This is one of the few *Glomus* species which has been shown to have tolerance to high Al and soil acidity (Barkdoll, 1987). *Glomus manihot* may really be adapted to acid soils. This fungus colonized *P. phaseoloides* when it was introduced into an acidic oxisol (Salinas et al., 1985).

It is not known how species of VA mycorrhizal fungi differ in their abilities to grow in acid soils. Abbott and Robson (1985) speculated that a VA mycorrhizal fungus which grows extensively intraradically may be less affected by soil acidity than one which grows extraradically. In this study, the acid-tolerant *Gl. manihot* LMNH 980 produced extensive intraradical structures including hyphae, vesicles, arbuscules, and spores in the roots of all four test forage legumes. Moreover, the same isolate had the highest spore germination in the Al tolerance assay done in soil apart from a host plant. This suggests that there are other mechanisms involved in the tolerance of VA mycorrhizal fungi to soil acidity and Al as speculated in Chapter III.

Acaulospora scrobiculata ASBC 456 and *E. colombiana* ECLB 356 did not consistently colonize *P. phaseoloides* and *S. guianensis*. However, when they did colonize, the level of root VA mycorrhizal colonization was high. This indicates that they have some degree of tolerance to Al and soil acidity. Mosse (1975) found that *Acaulospora laevis* was more effective in promoting plant growth in acid soils than in neutral soils. Thus, the failure of these isolates to colonize the legumes was likely due to inability of dormant spores to germinate, a phenomenon which is prevalent in the genera *Acaulospora* and *Entrophospora*. Barkdoll (1987) also encountered a similar problem with *A. longula* whose germination was very low although the isolate produced large

number of spores in pot culture; she also attributed this to a dormancy factor. The subject on spore dormancy of VA mycorrhizal fungi has been reviewed by Tommerup (1983).

The observed lower shoot P concentration of some mycorrhizal plants compared to *Rhizobium* control despite the increase in the growth of the former can be attributed to dilution effect. In general, the uptake of a limiting nutrient by a plant increases greatly with dry matter production and that the concentration of a limiting nutrient may decline with extremely small productions of dry matter (Steengjerg and Jakobsen, 1963). The test soil was P deficient. Although a small amount of P was supplied in the nutrient solution, part of this phosphate was rapidly fixed by Al^{3+} which saturates the exchange sites of the test soil almost completely. Due to dilution of P, shoot P concentration was not significantly correlated or even negatively correlated with shoot or root dry weight in most cases. However, shoot P concentration was highly correlated with root VA mycorrhizal colonization, in cases where P was not limiting such that dilution effect was minimal.

The overall shoot P concentration was low relative to what may be found in temperate food legumes. However, the values were not uncommmon in tropical forage legumes under field conditions because they have low external and internal P requirements. They can grow well at low soil available P or low shoot P concentration. In the present experiment, no

symptoms of P deficiency was observed in mycorrhizal plants. If the mineral composition of the forage does not meet the nutritional requirements of the animal to be fed, it is a common practice to provide inorganic mineral supplement in their ration. The latter is more practical and less costly than improving the P status of the legume by liming and fertilization.

Aluminum-tolerant VA mycorrhizal fungi improved the total amount of P taken up by the legumes in repeated experiments. Effectiveness in improving shoot or root P content of the hosts varied with fungal species and isolates. There was a high correlation between root VA mycorrhizal colonization and P content. Moreover, P content was highly correlated with spore germination at high Al. The tolerance of VA mycorrhizal fungi to Al directly affected the level of root VA mycorrhizal colonization which in turn, affected P nutrition.

Although the mechanisms for improved P nutrition were not investigated in this study, the growth of mycorrhizal fungi outside and inside the root seems significant. Both factors were related to Al tolerance as presented in Chapter III and in this study, respectively. Outside the root, the external hyphae aid in the absorption of available P (Rhodes and Gerdemann, 1975) and serve as a pathway for translocation of nutrients (Sanders and Tinker, 1971; Hattingh et al., 1973; Tinker and Sanders, 1975). Polyphosphate, synthesized by the fungal symbiont from inorganic P, is also translocated by

external and internal hyphae to the arbuscule (Cox et al., 1975; Callow et al., 1978; Cooper and Tinker, 1981). In the root, the active transfer of polyphosphate from the arbuscule to the host (Kinden and Brown, 1975) consequently results to the liberation of phosphates derived by the fungus from the soil.

The improvement in P nutrition by VA mycorrhizal fungi presumably led to enhancement of nodulation. Most isolates of VA mycorrhizal fungi which colonized *P. phaseoloides* and *S. guianensis* in the test soil improved the nodulation of the legumes in both trials. *Glomus manihot* LMNH 980 was usually the most effective in enhancing nodulation. This study demonstrated that the isolates which were effective in improving P nutrition were the ones effective in stimulating nodulation. Nodule number and nodule weight were highly correlated with shoot or root P content in all cases. This supports the conclusion forwarded by Newbould and Rangeley (1984) that VA mycorrhizal fungi increase nodulation and N_2 fixation of legumes primarily as an indirect effect of improved P nutrition. In fact, nodulated legumes have increased requirements for P (McLachlan and Norman, 1961) which affects both nodule initiation and nodule growth (Cassman et al., 1980).

Shoot and root N concentration were not always increased by VA mycorrhizal fungi. Analysis of the levels attained by plants colonized by different species and isolates of VA

mycorrhizal fungi suggests that N was a limiting nutrient in some experiments. Due to dilution of N, a weak or even negative correlation was obtained between N concentration and shoot or root dry weight in some experiments.

Shoot and root total N content were increased by Al-tolerant VA mycorrhizal colonization in all experiments. *Glomus manihot* LMNH 980 caused the greatest increase. Nitrogen content was consistently highly correlated with VA mycorrhizal colonization, with P content, and occassionally even with shoot or root P concentration. This implies that VA mycorrhizal colonization, through improved P nutrition of the legumes, contributed to N nutrition. This was also demonstrated in *M. atropurpureum* (Lynd et al., 1985). Phosphorus is important in N nutrition of legumes (Andrew and Robins, 1969). In the present study, shoot and root N content were highly correlated with nodule number and nodule weight which indicates that the legumes benefited from enhanced P nutrition indirectly through improved nodulation.

Since the correlation of N content with VA mycorrhizal colonization was greater than with nodulation, the benefit to legumes from improved P nutrition was more than just improved nodulation. Direct benefit from enhanced N_2 fixation process was also likely. Phosphorus improves N_2 fixation (Adu-Gyamfi et al., 1989). This is probably related to the high requirement of N_2-fixing systems for ATP which is essential for N_2 fixation (Bergersen, 1977; Pate, 1977).

It is often difficult to demonstrate improved growth of plants by VA mycorrhizal fungi in marginal soils, which are almost always acid, since most greenhouse and field experiments used *Gl. mosseae, Gl. etunicatum, and Gl. fasciculatum*. These species are widely distributed among VA mycorrhizal researchers and are relatively easy to mass produce in pot culture or aeroponic culture (Sylvia and Hubbell, 1986; Hung and Sylvia, 1988). However, all isolates of *Gl. mosseae* and *Gl. etunicatum* that were evaluated in the present studies were very sensitive to soil acidity and Al.

Those VA mycorrhizal fungi which demonstrated tolerance to soil acidity and Al improved the growth performance of the host legumes. Effectiveness in improving growth was, in fact, correlated with the relative degree of tolerance to soil acidity and Al evaluated in terms of spore germination, hyphal length, and mycelial growth index (Chapter III). Furthermore, the plant growth variables had high significant correlations with root VA mycorrhizal colonization.

The observed growth stimulation due to mycorrhizal colonization in this study is attributed to improved P nutrition and thus, nodulation, N_2 fixation, overall N nutrition of the legumes. High correlations between these variables were obtained. Appropriate VA mycorrhizal fungi permit plants to exploit nutrient-deficient soils (Black and Tinker, 1977; Hall, 1978).

In addition to the effects of VA mycorrhizal fungi on the legumes discussed so far, these fungi may have contributed in the ability of the legumes to tolerate high Al. Their hyphae or other structures may have a role in localizing Al inside or outside the root. A similar phenomenon may have occurred between VA mycorrhizal fungi and other metals. Copper and Zn concentration of mycorrhizal *L. leucocephala* were higher in roots than in shoots (Manjunath et al., 1989). In other studies, Fe and Mn concentration and content were lower in mycorrhizal plants than in nonmycorrhizal ones (Pacovsky et al., 1985; Pacovsky, 1986; Pacovsky et al., 1986).

The extent of root colonization by VA mycorrhizal fungi can be influenced by the host plant (Schenck and Kinloch, 1980). However, in the present study, there was no indication of host specificity even under such a condition of Al stress to both fungi and hosts.

The Al tolerance assay done in soil apart from the host plant (Chapter III) was able to select for VA mycorrhizal fungi that were effective in colonizing the legumes, and in improving their N-P nutrition, nodulation, and growth in a high Al acid soil. A similar assay procedure can be used in screening these fungi for tolerance to other soil inhibitory factors.

Inoculum density (e.g., spore number) is known to affect root mycorrhizal colonization level (Daft and Nicolson, 1969) and plant growth response to inoculation with VA mycorrhizal

fungi (Wilson, 1984; Haas and Krikun, 1985). Hence, it is important to standardize the inoculum density when comparing effectiveness among species and isolates (Daniels et al., 1981). In the present study, uniform number of mature spores that are free of hyperparasites and other visible defects was inoculated to the legumes. This was done instead of making inoculum potential (eg., most probable number) standard for all isolates, since the present study aimed to show the relationship between percent spore germination from the Al tolerance assay with the overall effectiveness of the fungi in a high-Al soil. A uniform inoculum potential would likely eliminate the inherent differences among isolates in their ability to colonize the legumes in a high-Al soil since the latter was correlated with spore germination in this soil.

Although the plant response experiments were done in steam-pasteurized soil, the results indicated that there may be a potential for field inoculation of VA mycorrhizal fungi in Pacolet soil. The most effective isolate in the present study was the one that originated from the test soil. However, the field population of the indigenous isolate *Gl. manihot* LMNH 980 in the field was very low. Plant growth response to field inoculation with VA mycorrhizal fungi when the population of native VAM is low has been obtained (Mosse, 1977; Hall, 1979; Abbott and Robson, 1982; Medina et al., 1988a). Since the indigenous isolate is infective, effective,

and has the ability to survive in this soil, the same isolate can be inoculated back.

It is important to consider, though, that the indigenous VA mycorrhizal fungi may become less adapted to its native site by soil amendments like liming and fertilization. Barkdoll (1987) obtained no growth response from the native VA mycorrhizal population in limed soil in the field. Similarly, Medina et al. (1987; 1988a) found that the native VA mycorrhizal fungi were less effective than introduced species in a limed, nonpasteurized soil under greenhouse conditions. This may not become a problem with *Gl. manihot* LMNH 980, which seems adapted to a wide range of soil acidity and Al. Its performance in the least Al-saturated Arredondo fine sand was comparable to that in its native Pacolet sandy clay loam.

The findings that Al is detrimental to VA mycorrhizal fungi, but that some isolates have tolerance to Al is of significant value in the utilization of these fungi in a pasture improvement program involving acid mineral soils which are rendered unproductive by high percent Al saturation. *Glomus manihot* LMNH 980, *Gi. gigantea* GGGT 109, *Gi. margarita* GMRG 185 and GMRG 444 are Al tolerant and are effective in improving N and P nutrition, nodulation, and growth of forage legumes in a highly Al-saturated soil. These isolates, which are available from INVAM, are good potential candidates in such a pasture improvement program.

CHAPTER V
SUMMARY AND CONCLUSIONS

Several VA mycorrhizal fungi (24 isolates representing 15 species) were evaluated for tolerance to soil acidity and Al based on spore germination and hyphal growth in acid soils with varying levels of exchangeable Al and percent Al saturation. Pacolet sandy clay loam with pH 4.3 had 157 mg kg^{-1} Al and 100% Al saturation, Wauchula sand with pH 5.0 had 30 mg kg^{-1} Al and 37 % Al saturation, and Arredondo fine sand with pH 4.5 had 14 mg kg^{-1} Al and 12% Al saturation. Selected isolates of VA mycorrhizal fungi which vary in the degree of tolerance to Al were further evaluated for effectiveness to colonize *P. phaseoloides* and *S. guianensis*, and improve the host P nutrition, nodulation, N nutrition, and growth in 100% Al-saturated Pacolet sandy clay loam. Moreover, the most effective isolate was further tested in the same manner for *L. leucocephala* and *C. pubescens* under similar Al stress. Sensitive isolates were acclimatized to soil acidity and Al by culturing them in soils with progressively increasing soil percent Al saturation, and their performance after the acclimation period was evaluated.

There were interspecific and intraspecific variations in the tolerance of VA mycorrhizal fungi to soil acidity and Al

Glomus species were extremely sensitive except *Gl. manihot* LMNH 980, the isolate indigenous to Pacolet sandy clay loam, whose spore germination was unaffected by Al and whose hyphal growth was reduced only at 100% Al saturation. *Glomus mosseae* LMSS 156, LMSS 313, and LMSS 378 failed to germinate in all of the test soils suggesting that spore germination of this species was prevented at an Al saturation of at least 12%. Spore germination and hyphal growth of *Gl. etunicatum* LETC 236, LETC 329, and LETC 455 was reduced starting at 37% Al saturation and was prevented at 100%. Although *Gl. clarum* germinated at 100% Al saturation, its spore germination and hyphal growth was reduced starting at 37%. *Acaulospora scrobiculata* ASBC 456 had consistently low germination in all test soils particularly in Wauchula sand. The other *Acaulospora* species tested, *A. appendicula* AAPD 130, *A. spinosa* ASPN 257, ASPN 629, *A. longula* ALGL 316, ALGL 652, as well as the *Entrophospora* species *E. colombiana* ECLB 356 and *E. schenckii* ESHK 383 failed to germinate in all the test soils. The response may be due to spore dormancy which is prevalent in these genera, rather than lack of tolerance to soil acidity and Al. Most species of *Gigaspora* and *Scutellispora* had high tolerance to Al stress. Spore germination of *Gi. margarita* GMRG 444, *S. heterogama* CHTG 139, *S. calospora* CCLS 348, and *S. pellucida* CPLC 288 as well as the hyphal growth of GMRG 444, *Gi. gigantea* GGGT 663, and CCLS 269 were not affected by Al. The other isolates were even

favored by Al. Spore germination of *Gi. margarita* GMRG 185 and the hyphal growth of *Gi. gigantea* GGGT 109 increased with increasing percent Al saturation. *Scutellispora* species were less tolerant than *Gigaspora* species. Spore germination of *S. calospora* CCLS 269 and hyphal growth of *S. heterogama* CHTG 139 and *S. calospora* CCLS 348 were reduced at 100% Al saturation. *Scutellispora pellucida* CPLC 288 whose growth was reduced starting at 37% Al saturation was the least tolerant in the genus. Furthermore, isolate differences in the degree of Al tolerance were observed between *Gi. margarita* GMRG 185 and GMRG 444, between *Gi. gigantea* GGGT 109 and GGGT 663, and between *S. calospora* CCLS 348 and CCLS 269, where the former isolates were more tolerant than the latter.

Comparing the performance of different VA mycorrhizal fungi in a 100% Al-saturated soil, *Gl. manihot* LMNH 980 had the highest spore germination while *Gi. gigantea* GGGT 109 had the most extensive hyphal growth and greatest mycelial growth index among 14 isolates evaluated representing 9 species. None of the *Gl. etunicatum* or *Gl. mosseae* isolates germinated and *Gl. clarum* LCLR 551 had very low germination. Intraspecific variations were again apparent between isolates of *Gi. margarita, Gi. gigantea,* and *S. calospora* in terms of one or more of the following: spore germination, hyphal growth, and mycelial growth index. *Glomus manihot* LMNH 980 produced shorter hyphae than most *Gigaspora* and *Scutellispora* species. Relative to the other VA mycorrhizal fungi, *Gi.*

margarita had consistently high spore germination, hyphal growth, and mycelial growth. The symbiotic mycorrhizal association was successfully established in the forage legumes *P. phaseoloides, S. guianensis, L. leucocephala,* and *C. pubescens* grown in a 100% Al-saturated Pacolet sandy clay loam. In the first two hosts where several species and isolates of VA mycorrhizal fungi were evaluated, interspecific and intraspecific variations existed in the ability of the fungi to colonize the legumes and in the level of root VA mycorrhizal colonization produced. *Glomus etunicatum* LETC 236, LETC 329, LETC 455, and *Gl. mosseae* LMSS 378 which were found very sensitive to soil acidity and Al (Chapter III) failed to colonize either *P. phaseoloides* or *S. guianensis* under Al stress (Chapter IV). Several isolates including *Gl. manihot* LMNH 980, *Gi. gigantea* GGGT 109, GGGT 663, *Gi. margarita* GMRG 185, GMRG 444, *S. heterogama* CHTG 139, and *S. pellucida* CPLC 288 consistently colonized the legumes. Among these isolates, *Gl. manihot* LMNH 980, originally isolated from Pacolet sandy clay loam, colonized most extensively. In general, *Gigaspora* species had greater root colonization than *Scutellispora* species. Legumes colonized by *Gi. margarita* 185, GMRG 444, and *Gi. gigantea* GGGT 109 had higher percent mycorrhizal colonization than those colonized by *S. heterogama* CHTG 139, *S. calospora* CCLS 348, and *S. pellucida* CPLC 288. Comparing isolates of the same species, colonization by *Gi. gigantea* GGGT 109, *Gi. margarita* GMRG 444, and *S. calospora*

CCLS 348 was higher than that by GGGT 109, GMRG 185, and CCLS 269, respectively. *Acaulospora scrobiculata* ASBC 456 and ECLB 356 did not consistently colonize the legumes probably due to spore dormancy rather than lack of Al tolerance.

Not all VA mycorrhizal fungi which colonized the legumes improved the shoot P concentration of the host due to dilution effect. For instance, the shoot P concentration of plants colonized by *Gl. manihot* LMNH 980 was not different or even lower than that of the *Rhizobium* control despite the increase in growth of the former. Moreover, the shoot P concentration of *S. guianensis* in trial 2 was not improved by VA mycorrhizal fungi; thus, shoot P concentration was not correlated or even negatively correlated with shoot or root dry weight. In cases where P dilution was not that pronounced as in *S. guianensis* trial 1, colonization by all isolates of VA mycorrhizal fungi improved the shoot P concentration of the host. In this trial, *Gl. manihot* LMNH 980 which colonized the host most extensively increased shoot P concentration most while *S. pellucida* CPLC 288 had the least contribution to improvement of shoot P concentration. Consequently, there was a fairly high correlation between shoot P concentration and root VA mycorrhizal colonization. The root P concentration of *P. phaseoloides* in both trials and of *S. guianensis* in trial 1 was increased by mycorrhizal colonization. The effect of *Gl. manihot* LMNH 980 and *Gi. gigantea* GGGT 109 on root P concentration of both hosts was usually better than *A.*

scrobiculata ASBC 456, *S. heterogama* CHTG 139, and *S. pellucida* CPLC 288 in repeated experiments. There was a fairly high correlation between root P concentration and root VA mycorrhizal colonization in *P. phaseoloides* trial 2.

Mycorrhizal fungi repeatedly improved the total amount of P taken up by *P. phaseoloides* and *S. guianensis*. In both trials, *Gl. manihot* LMNH 980 and *Gi. gigantea* GGGT 109 were the most effective while *S. calospora* CCLS 348 and *S. pellucida* CPLC 288 were the least. Although root VA mycorrhizal colonization by *Gi. margarita* GMRG 444 was always higher than GMRG 185 in both hosts, the latter was more effective in improving shoot and root P contents of *P. phaseoloides* in trials 1 and 2 and root P content of *S. guianensis* in trial 1. Nevertheless, there was a high correlation between root VA mycorrhizal colonization and shoot or root P content.

The improvement of host P nutrition by VA mycorrhizal fungi presumably led to enhancement of nodulation. Nearly all isolates of VA mycorrhizal fungi which colonized *P. phaseoloides* and *S. guianensis* improved the nodulation of legumes in both trials. Isolates which were effective in improving P nutrition were the ones effective in stimulating nodulation. For instance, *Gl. manihot* LMNH 980 was usually the most effective in enhancing nodulation whereas, *S. calospora* CCLS 348 and *S. pellucida* CPLC 288 stimulated nodulation of *P. phaseoloides* in only one trial. Nodule

number and nodule weight were always highly correlated with shoot or root P content.

Shoot and root N concentration were not always increased by VA mycorrhizal fungi due to dilution of N which was also a limiting nutrient in this study. Consequently, shoot or root N concentration was only weakly, if not negatively or insignificantly, correlated with shoot or root dry weight.

On the other hand, shoot or root total N content were consistently increased by VA mycorrhizal fungi. In all cases, *Gl. manihot* LMNH 980 caused the greatest increase. *Scutellispora* species were usually found less effective than *Gigaspora* species. Shoot and root N content was consistently highly correlated with shoot and root P content as well as with nodule number and nodule weight. Root VA mycorrhizal colonization contributed to N nutrition of legumes through improvement of host P nutrition and consequently, nodulation.

All four forage legumes tested showed significant growth responses to VA mycorrhizal colonization. Shoot and root fresh and dry weights of host plants were consistently improved by *Gl. manihot* LMNH 980, *Gi. gigantea* GGGT 109, *Gi. margarita* GMRG 444, GMRG 185 and *A. scrobiculata* ASBC 456. Isolates *S. heterogama* CHTG 139, *S. calospora* CCLS 348, and *S. pellucida* CPLC 288 increased some but not all parameters. There was no response to inoculation with *Gl. etunicatum* LETC 236, LETC 329, LETC 455, and *Gl. mosseae* LMSS 378 as these isolates failed to colonize the legumes due to soil acidity

and Al. The growth parameters were highly correlated with root VA mycorrhizal colonization, N content, nodulation, and P content.

The studies demonstrated clearly that the degree of Al tolerance of VA mycorrhizal fungi, evaluated in terms of spore germination and hyphal growth in soils with varied levels of exchangeable Al and percent Al saturation, affects the extent of their host root colonization and, consequently, their effectiveness in improving P nutrition, nodulation, N nutrition, and growth of host forage legumes in an acid mineral soil.

Moreover, the results indicated that development of Al tolerance by isolates of VA mycorrhizal fungi which are naturally sensitive to this factor is possible by acclimation. A third of the Al-sensitive isolates were able to germinate and grow in a 100% Al-saturated soil, but percent spore germination was very low and hyphal growth was minimal.

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BIOGRAPHICAL SKETCH

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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